Ecological and Biochemical Adaptation in Four Coexisting Species of the Spider Genus META (Tetragnathidae).

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Abstract.

Differentiated enzyme loci are valuable diagnostic characters for juveniles of closely related spider species, and adults of sibling species. The genetics of seven enzyme loci in Meta examined by starch gel electrophoresis are discussed. Four of these loci were used for routine identification of M. mengei, M. segmentata, M. merianae and M. menardi. These species are ranked along an axis of genetic similarity in the above order.

The in vivo deployment of the metabolic enzymes which were active on zymograms is discussed: spiders depend on these enzymes for NAD^+ recycling during rapid muscular activity. Interspecific allozyme differences are discussed in relation to temperature compensation at the enzyme level. Thermal modulation of LDH and MDH Km's were demonstrated, but no significant inter-allozyme difference was detected.

Meta species leave their eggsacs in the 2nd instar and reach maturity in the 7th instar. M. segmentata hatches in May and mates in the following September. M. mengei hatches in July, matures 14 months later, and mates in April. The events of M. merianae's life cycle follow 1-2 months behind M. mengei's. Young M. menardi disperse into the field layer in April-May, moult once, and return to "microcaves" where they mature and mate, 13-14 months after hatching.

It is argued that large spider instars are more efficient predators and more sensitive to competition than smaller conspecifics. Population size is limited mainly by food supply and the negative influence of adult female density on fecundity. Consequently, adult Meta stages have diverged along the horizontal space and breeding season niche dimensions, although juveniles overlap spatially and temporally. Large instars are also more vulnerable to attack by visual predators than smaller conspecifics, and adaptations for predator avoidance have sharpened habitat differences between the species and caused divergence along the diel time dimension.

Acknowledgements.

This research was undertaken under the supervision of Dr. N. P. Ashmole, to whom I am grateful for encouragement and support. I would also like to express my thanks to Dr. C. H. Sissons for instruction in enzymological techniques, and to Susan R. Auld for permission to use data from her BSc. thesis.

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Chapter 1. The genus META.

The researches reported in this thesis focus on four sympatric orb web spider species. The genus to which they belong is represented globally by over sixty species, but only five are native to Western Europe (Bonnet 1957). These are Meta mengei (Blackwall), M. segmentata (Clerk), M. merianae (Scopoli), M. menardi (Latreille), and M. bournetti Simon.

M. menardi and M. bournetti are cave-dwelling spiders. M. bournetti is confined to a narrower, more southerly geographical distribution than its close relative and rarely occurs in Britain. This species was not found during the present study. M. menardi, on the other hand, is widespread throughout Britain and Europe and its range extends from North Africa to Northern Scandinavia. This species also occurs in North America (Levi pers. com.) and Japan (Yaginuma 1958). The adult M. menardi's cephalothorax and legs are dark brown and the tapering abdomen is shiny black with only faint dorsal markings. Adult females may exceed 13mm in body length. Figure 1.1 illustrates four stages in the life cycle of M. menardi.

M. merianae also occurs in caves but the true home of this species is at the transition from exposed field layer vegetation to dark cave-like situations. (The field layer is broadly defined as the zone 10cm - 2m between the ground and lower canopy layers.) Adult M. merianae are commonly found in sheltered damp places occupying web sites under overhangs formed by rocks, peat, or the exposed roots of vegetation on slopes, frequently in the vicinity of water. This species is common throughout Europe from the Mediterranean to Northern Scandinavia and it has also colonised Iceland and the Faroe Islands.

Figure 1.2 illustrates adult M. merianae and adult M. mengei.

Figure 1.1 Four stages in the life cycle of M. menardi.

Top left and right: dorsal and ventral views of a 3rd instar juvenile, approximately X5. Unusually, juvenile M. menardi are not cave-dwellers in Scotland.

Centre: a 5th instar (estimate) juvenile at the hub of its web. The spider is about 1m from the camera lens in a "microcave" formed by rocks, and is approximately life size.

Below: an adult female M. menardi photographed while eating her old web. The spider is about 2.5m from the camera lens in an enclosed chamber formed by large rocks and the ground. The bleached part of the photograph is due to the electronic flash light reflecting off the roof at the narrow entrance of the microcave. At either side of the adult female the empty eggsacs of previous generations of adults hang from the roof. The spider and eggsacs are approximately life size.

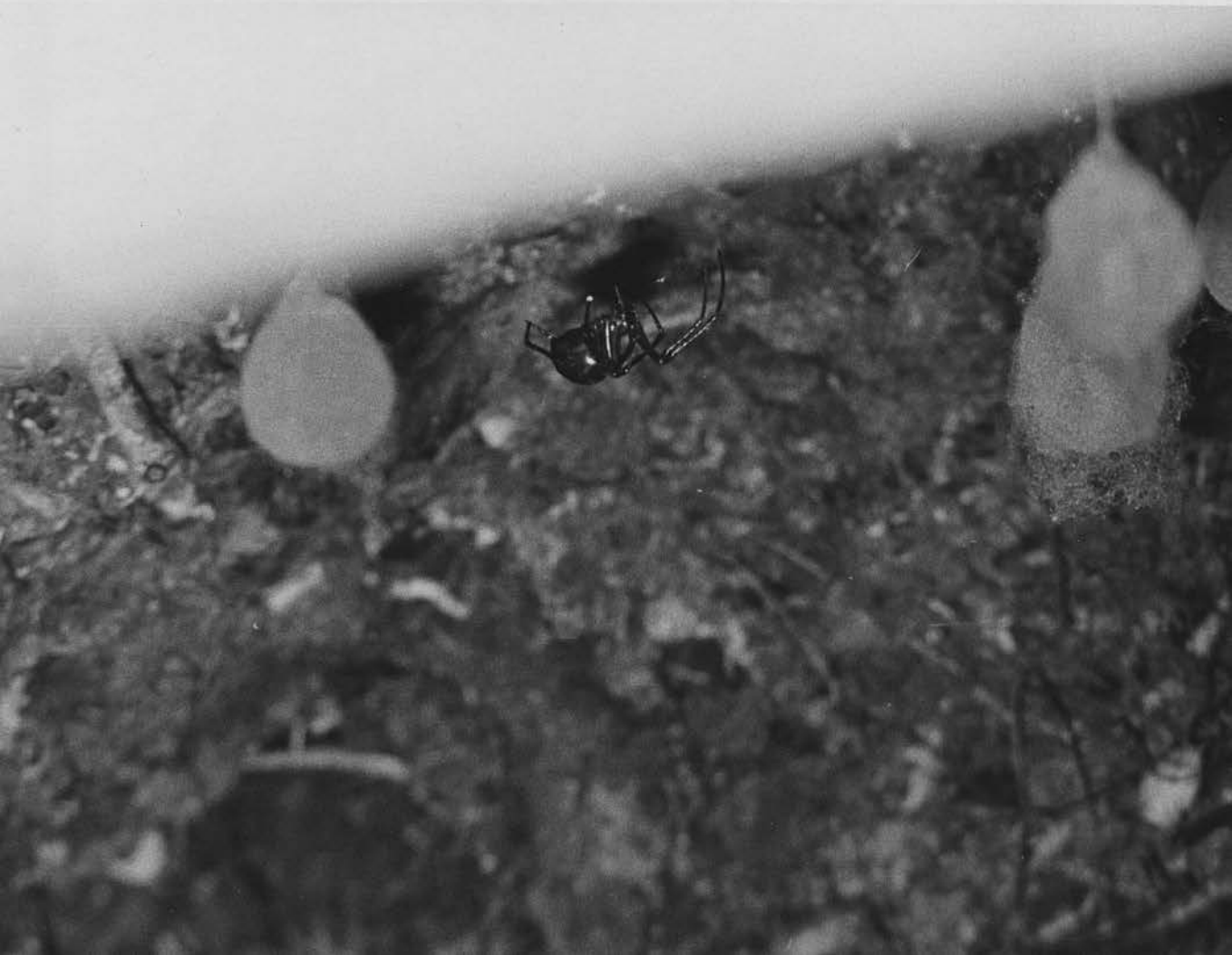


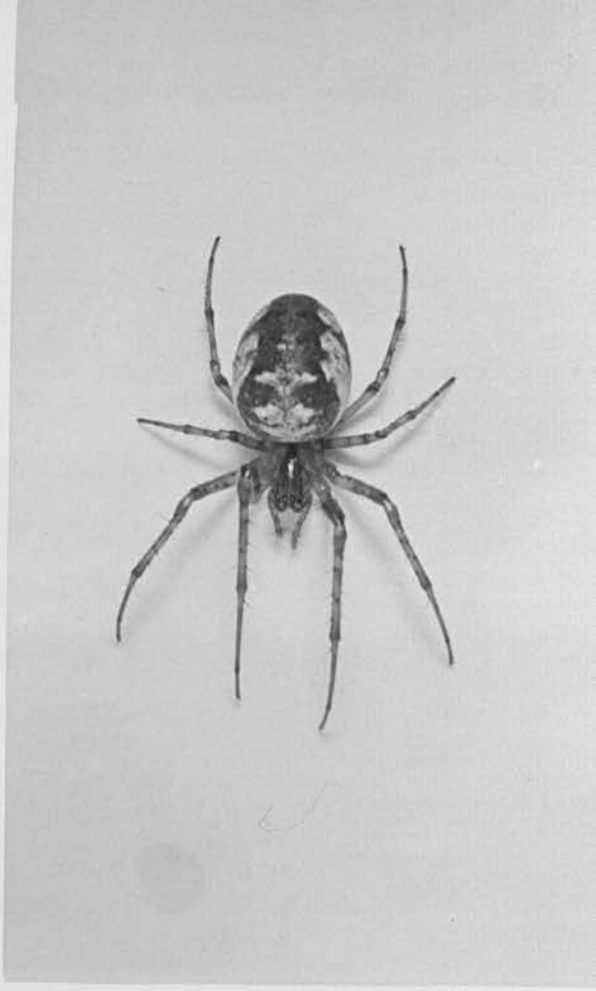
Figure 1.2 Adult *M. merianae* and *M. mengei* (X5)

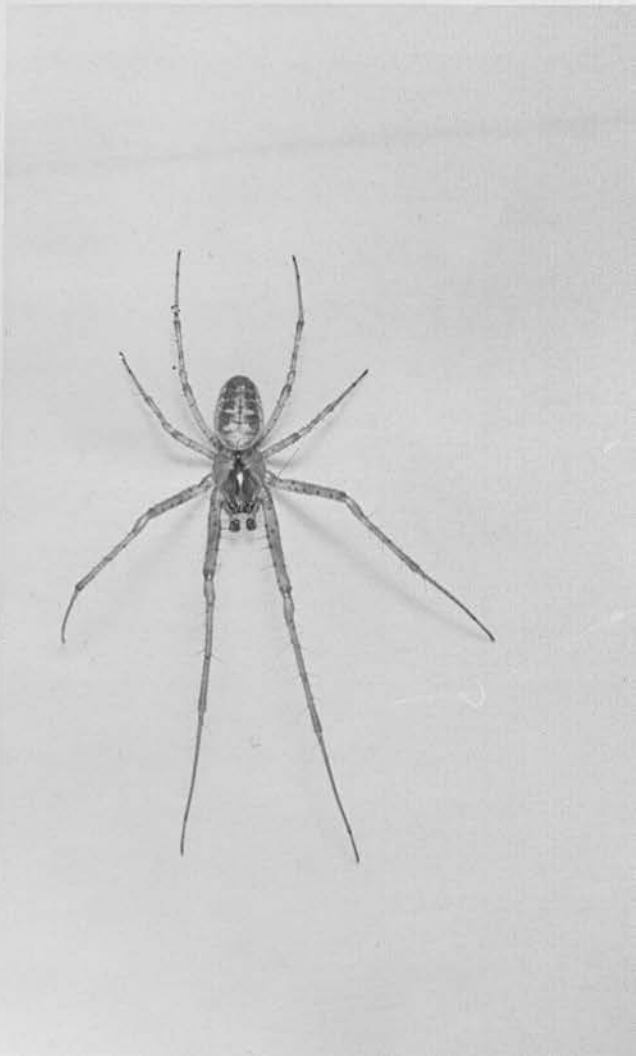
Adult female *M. merianae*

Adult female (gravid) *M. mengei*

Adult male *M. merianae*

Adult male *M. mengei*





Adult female M. merianae are normally between 5.5-9mm long and by comparison, M. mengei is the smaller species, reaching adult female body lengths of about 4-6mm. M. segmentata is a morphological sibling species of M. mengei and is generally slightly larger. The cephalothorax and legs of M. mengei have fewer and less dense black markings than M. merianae have, and the black and white photographs show that the dorsal patterns of the two species are similarly formed but differ markedly in pigment contrasts. The colours of M. merianae's dorsal pattern mainly lie in the tonal range from light brown to black, and in M. mengei and M. segmentata the pattern is composed of shades of white, greens, yellows, reds, browns, and black. In M. mengei a contrasty reddish-brown and cream pattern is the rule, but in M. segmentata there are many more tint and contrast variations on this theme. Figure 1.2 shows also that the abdomens of male Meta are smaller and their legs longer than the females', but there is no sex dimorphism in the shape or size of the cephalothorax.

M. mengei and M. segmentata are the commonest British orb web spider species (Bristowe 1958). They live in the field layer vegetation of deciduous woodland, hedgerows, and the shaded edges of meadows. The major ecological difference between them is season of breeding: M. mengei breeds in spring and early summer and M. segmentata breeds in autumn. Throughout their known geographical ranges these siblings co-occur. They are found from the Mediterranean to Northern Scandinavia and east into the USSR. M. segmentata has also been recorded in the Far East, but specimens of Japanese "M. segmentata" kindly sent to me by Prof. T. Yaginuma are quite unlike the European species in size, colouration, and sex organ morphology.

To the inexperienced eye only the adult males of these two sibling species are clearly distinguishable. M. segmentata was first described by Clerk in 1757 and in 1866 Menge distinguished a

"smaller variety". Four years later, Blackwall regarded this variety as a separate species and named it M. mengii. Since that time, however, M. mengei has usually been regarded as the spring brood of M. segmentata and omitted fomm species lists (eg. Bristowe 1939, 1958), of has been given the status of subspecies (eg. Locket and Millidge 1953; Bonnet 1957). Chrysanthus (1953) settled this debate by showing that the internal sex organ morphologies of the two species are non-overlapping and distinct. He confirmed the species status of M. mengei and this is now widely accepted (Locket, Millidge, and Merrett 1974).

Meta species were formerly grouped with the largest British orb-weaving family, the Araneidae (10 genera, 36 species), but it is currently thought that their proper place is with the Tetragnathidae (3 genera including Meta, 14 species) (Locket, Millidge, and Merrett 1974). Meta sex organs lack the complexity of araneid sex organs and bear a closer resemblance to those of tetragnathid spiders. The mouthparts of Meta and Tetragnatha are also similar, and Meta webs are open-hubbed, which is characteristic of Tetragnatha webs. However, the Araneidae and the Tetragnathidae are phylogenetically related, unlike the Uloboridae which arrived at the orb web by convergent evolution (Kullman 1972). The Tetragnathidae diverged freom the main araneid stock at an early stage in its evolution (Bristowe 1958), and the true position of Meta, in terms of taxonomic and genetic distances, may lie between these two families.

The four chapters which follow span diverse topics. The starting point was with the colour pattern variations of M. segmentata

Casual observations had suggested that the tints and contrasts of these spiders' dorsal patterns tend to match the vegetation on which they spin their webs and that adjacent plots of vegetation contain spiders which, on average, are coloured quite differently. Invertebrate populations are generally more genetically heterogeneous than vertebrates are and one explanation for this is that small immobile animals experience their environments as sets of alternatives, with the result that local adaptations evolve (Selander and Kaufman 1973). In the present context, vegetation types (grasses, gorse bushes, bracken for example) are environmental alternatives. Despite the fact that the young of many spider species disperse at random with respect to suitable substrates by ballooning in air currents (Duffey 1956), behaviour which is bound to undo whatever genetic differentiation took place between previous adult generations in alternative microhabitats, I felt that a study of M. segmentata's enzyme polymorphisms in isolated island communities might prove rewarding.

The results of a pilot study conducted on the islands and shores of Loch Sween, Argyll, in October 1973 turned up nothing exciting as far as local adaptation in M. segmentata was concerned, but they unexpectedly showed that adult specimens of M. mengei, which had been overlooked during examination under the microscope, were genetically quite distinct from M. segmentata. The enzyme loci were more reliable as diagnostic characters than were conventional morphological characters. Chapter 2 describes these results and the differences subsequently found between the sibling species and M. menardi and M. merianae, and Chapter 3 describes an avenue of research which stemmed directly from these results. Chapters 4 and 5 deal specifically with the life histories of Meta species in Scotland

and the factors which may have influenced the evolution of the spatial and temporal niche differences between them. This part of the research too stemmed from the findings of the October 1973 pilot study, as the introductory paragraphs of Chapter 4 explain.

Chapter 2. A Biochemical Method for Species Identification.

Gel electrophoresis involves the separation of soluble proteins by their charges and molecular weights. A voltage is applied across a buffered "molecular sieve" of starch or polyacrylamide gel in which the protein samples are suspended, and because charged particles move in an electric field the proteins migrate towards the electrodes at a rate which is proportional to their net charge / molecular weight ratios. The net charge of a protein molecule depends upon the number, types, positions, and isoelectric points of ionising groups and the pH and temperature of the medium. Approximately 30% of amino acid substitutions can alter the net charges and hence electrophoretic mobilities of proteins (Lewontin 1974).

Proteins migrate across the gel in bands and are stained in situ after electrophoresis. Structural proteins or, more often, reaction specific enzymes can be stained separately. The gel or a thin slice of the gel is bathed in a solution of buffer, enzyme substrates, and a dye which couples either directly or through intermediates with the reaction products and precipitates on the gel at the site of catalytic activity. Separation of enzymes by electrophoresis followed by selective staining is called the zymogram technique (Wagner and Selander 1973).

Two kinds of electrophoretically detectable structural variants are recognised. Enzymes which have similar catalytic functions but which differ structurally are called isozymes, and isozymes which are allelic variants segregating in a population are called allozymes (Prakash, Lewontin, and Hubby 1969).

Electrophoresis is a valuable tool for examining genetic heterogeneity in populations and the genetics of speciation. For example, comparisons of allozyme frequencies and allozyme identities

at polymorphic loci, mainly in Drosophila, show that geographically separated populations within species are remarkably similar (Prakash, Lewontin, and Hubby 1969) and that from semispecies (Richmond 1972; Selander, Hunt, and Yang 1969), through sibling species (Ayala and Powell 1972), to indirectly related congeners, morphological and ecological divergence is loosely paralleled by the amount of inter-deme differentiation at enzyme loci. However, recent data indicate that there are much greater degrees of genetic differentiation between closely related congeners than had previously been recognised (Coyne 1976; Singh, Lewontin, and Felton 1976). Electrophoresis has revealed genetic divergence and reproductive isolation between previously undetected morphological sibling species (Webster and Burns 1973), and in populations of known genetic constitution allozyme genotypes can be used to establish species identities where diagnosis by morphology fails (Ayala and Powell 1972). In the present study the zymogram technique was used for this very purpose.

Methods.

The study area and sampling methods will be described in detail in Chapter 4. Spiders were collected from the Argyll study area either by sweep-netting or individually from their webs. They were kept alive in 6 X 1.5cm clear plastic tubes for from 12 hours to 3 days until my return to Edinburgh, whereupon the samples were stored at below -20°C in a deep freeze.

Samples were separated into species using morphological characters wherever that was possible, but in a large number of cases - small juveniles and most instars of M. mengei and M. segmentata in particular - spiders could not confidently be assigned to species and these were prepared for enzyme electrophoresis.

Each specimen was homogenised in a well of a 3-well glass slide on ice with 1-2 volumes of distilled water and homogenates were drawn off with 5mm squares of Whatman no.3 chromatography paper (inserts). Two samples were taken from each homogenate, one for immediate use and the other for future reference. Initially, both were used to screen a broad sample of enzymes, one insert on each of two gels. Stored inserts were kept in the numbered wells of haemagglutinin trays in the deep freeze. Later, 24 of these squares were inserted perpendicularly into each 20 X 15 X 0.65cm horizontal starch gel along the origin line, 5cm from the cathode. Tris-Citrate pH 6.2 and Tris-EDTA-Borate pH 8.6 gels were made up 36-24 hours before each experiment and cooled to 6°C immediately beforehand. Details of the gel and electrode buffers appear in Appendix 1. The electrode buffers were connected to the gels in the cold-room with Whatman no.1 chromatography paper wicks (10 thicknesses), and bromophenol-blue on the 25th inserts marked the progress of the buffer boundaries. Voltage was applied with a VoKam power pack and the Tris-Citrate and

Tris-EDTA-Borate gels were run at constant currents of 60mA and 40mA for 2 and 4 hours respectively. Gels were sliced horizontally into 4 parts and each slice was incubated in 50ml staining solution in a plastic instrument tray for 15 minutes to 1 hour at room temperature. When the activities were sufficiently stained, the relative band positions were recorded and the gel slices were fixed in methanol: acetic acid: water solution (4:1:4) and sealed in clear plastic bags for short-term future reference.

The two gel systems were initially used to screen for polymorphisms at 17 enzyme loci which are commonly used in population studies. These are listed in Appendix 1. However, only six enzyme systems resolved into clear active bands with sufficient repeatability for genetic interpretations to be made of them. These were non-specific esterases, malate dehydrogenase (MDH), lactate dehydrogenase (LDH), glutamate-oxaloacetate transaminase (GOT), α-glycerophosphate dehydrogenase (α-GPDH), and phosphoglucose isomerase (PGI). Esterase, PGI, and α-GPDH assays were discontinued for reasonswhich are explained in the Results section of this Chapter, and the remaining assays were all made with slices from Tris-Citrate gels. The stain recipes for these six enzymes appear in Appendix 1.

Results and Discussion.

The October 1973 pilot study sample consisted of 180 adult and 9 juvenile Meta, individually collected from webs. When the results of the first two gels (48 spiders) were examined it was immediately apparent that genetic variation was present at the esterase, GOT, LDH, and MDH loci, but that, unexpectedly, the genotypes were bimodally distributed. That is, the variants at none of the loci were in Hardy-Weinberg equilibrium and the sample fell neatly into two genotype groups, approximately in the ratio 3:1, with very few heterozygotes. Spiders which were deviant at one locus would be deviant at all loci. Closer examination of the remaining adult males confirmed that M. mengei were present in the mainly adult M. segmentata sample and that the two alternative genotype groups corresponded exactly with the two species. At that time I was unaware of Chrysanthus' (1953) paper, but this result independently convinced me that these genetically discontinuous co-occurring adult populations could only be true sexually and, presumably, ecologically isolated species. Thus the spiders' genotypes indicated their species identities, and if several loci were screened simultaneously - in order to avoid errors of species assignment introduced by the existence of polymorphisms within species - genetic data could be collected for each locus as if species diagnosis was independent of the genotype at that particular locus. The interpretations which follow were worked out using this procedure.

Figure 2.1 illustrates the esterase polymorphisms found at two homologous loci in M. mengei and M. segmentata, and Table 2.1 reports the gene frequencies observed at these loci in the October 1973 sample. In M. segmentata there are four faintly staining Est-1 allozymes, Est-1a, b, c, and d, and two of these, Est-1c and d are

Figure 2.1 Esterase allozymes at two polymorphic loci in M.mengei and M.segmentata.

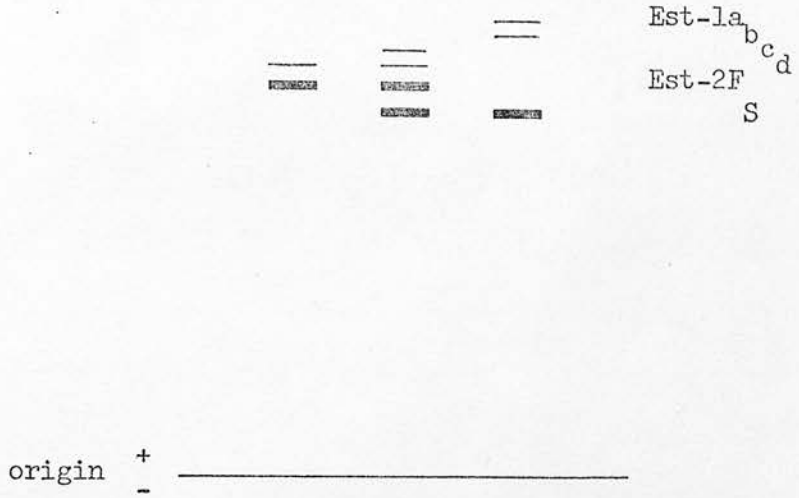


Table 2.1 Esterase gene frequencies in October 1973.

		Est-1				Est-2	
	N	a	b	c	d	F	S
M.mengei	39	--	--	0.279	0.721	0.936	0.064
M.segmentata	150	0.014	0.068	0.295	0.007	0.01	0.99

Table 2.2 Expected Est-2 genotype frequencies.

	Est-2F	Est-2FS	Est-2S
M.mengei	0.876	0.12	0.004
M.segmentata	0.0001	0.0198	0.98

shared by M. mengei. The Est-1c allele is at virtually the same frequency in these two sibling species. Since this is the most heterogeneous locus of seven loci examined in Meta (alleles segregate at neither very high nor very low frequencies), it is the most useful for comparing isolated communities of the same species (the sampling variance of the binomial distribution equals pq/n). The October 1973 sample showed that the M. segmentata populations on Loch Sween islands are genetically continuous.

Est-2 bands are more strongly staining than Est-1 bands, and this 2-allele locus is more differentiated between the siblings. Differentiated loci at which different alleles are at high frequencies in two species can be used as diagnostic characters: each individual is assigned to the species in which its genotype is most frequent. Here, the proportion of overlap (Ayala and Powell 1972) of expected Est-2 genotype frequencies is 0.001 (M. segmentata Est-2F) + 0.0198 (M. segmentata Est-2FS) + 0.004 (M. mengei Est-2S) = 0.0239. The probability of error in assigning Est-2S genotypes to M. segmentata or Est-2FS and Est-F genotypes to M. mengei is $\frac{1}{2}$ of this; ie. 0.012. This locus therefore falls short of the criterion "diagnostic at the 0.01 level", but if morphology and Est-1 allozymes are also taken into account, Est-2 genotypes are satisfactory characters for separating M. mengei from M. segmentata.

Esterase assays were, unfortunately, of limited value in later research because I was unable to identify the homologues of these loci in M. merianae and M. menardi, whose esterase bands were also less clear. The latter species were first found as juveniles in field layer sweep samples, and in the case of M. menardi, I kept records of cephalothorax lengths and enzyme phenotypes for several months before confirming the identities of these juveniles by comparison with

an adult female's enzyme phenotypes. Transferring data from fuzzy zymograms into numerical representations or drawings involves making on-the-spot decisions about the identities of allozymes. It helps to know what alternatives one might have, and if the biology of the organism precludes systematic breeding experiments, it is virtually impossible to make such interpretations when band and locus homologies are not immediately apparent. Thus continuing with esterase assays ceased to be worthwhile; and for the same reason a polymorphic MDH band system, shown as monomorphic in Figure 2.2, and a monomorphic α-GPDH locus were not routinely recorded. This left a sample of four loci which were of use in species identification.

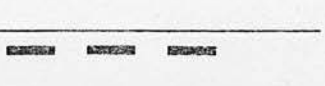
The loci for which data were collected routinely are illustrated in Figures 2.2 - 2.5. Observed phenotype frequencies and simplest plausible genotypic interpretations appear below each column of bands. Sample sizes are based on well-defined preparations: in many cases one or two gel slices did not stain clearly and these had to be rejected, although re-running the gel with the second set of stored inserts often filled such gaps in the data. This did not matter much in practice, because with three or four enzyme assays per spider there was redundancy in the identification method. Genotypic interpretations are speculative because the genetic bases of the phenotypes are untested and there is probably hidden variation within band positions. Here I present interpretations which account for the observed variation with the fewest alleles, but several other interpretations may be equally plausible.

The patterns of four or six equally spaced anodal MDH isozymes (Figure 2.2) represent a series of subunit aggregations thus: monomer, dimer, ... , hexamer. The MDH subunit is structurally differentiated among the species, except in M. segmentata and M. merianae,

Figure 2.2 MDH phenotype frequencies in Meta , and assumed genotypes.

anodal MDH

origin + −



cathodal MDH

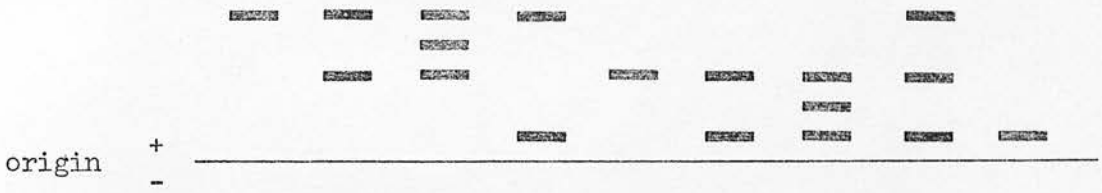
Phenotype	MDH1	MDH2	MDH3	N
Genotype	aa	bb	cc	
M.merianae	1.0	--	--	298
M.segmentata	1.0	--	--	233
M.menardi	--	1.0	--	206
M.mengei	--	--	1.0	569

which share the same MDH phenotype (MDH1). The M. mengei subunit differs from this by charge (MDH3), and the M. menardi subunit binds only in four sizes of aggregation (MDH2). The cathodal MDH locus possibly represents mitochondrial MDH activity, as it does in Limulus (Selander et. al. 1970), although spider tissues normally exhibit low activities for mitochondrial enzymes (Linzen and Gallowitz 1974). Within species, anodal phenotypes appeared to be monomorphic, although unidentified factors influenced the activities of MDH isozymes (or degrees of dissociation) and the precice number of clearly resolved lighter (faster) molecular weight isozymes on individual gel slices. The anodal phenotypes closely resemble those of Limulus (Selander et. al. 1970).

GOT is a dimeric enzyme which forms 2- and 3-banded heterozygous phenotypes, each sharing the same parent band positions (Figure 2.3). GOT1, GOT3, and GOT5 therefore each represent the band positions of two allozymes which do not separate by charge but which can be distinguished by their abilities to hybridise with other allozymes: GOT2, GOT3, and GOT4 are hybrid bands in some heterozygotes. Some cathodal GOT activity was observed on most gels, but the bands were too indistinct to score. In my interpretation of GOT genotypes I assume that the rarer alleles within band positions did not occur in homozygotes and the identities of alleles shared between species has been decided on the basis of phenotype frequencies rather that heterozygote band morphologies.

LDH is also a dimeric enzyme which forms 2- and 3-banded phenotypes in heterozygotes (FIgure 2.4). In addition, there is the complication of minor LDH band activity which was observed on some preparations. For convenience of presentation, these are illustrated only for the LDH3 and LDH4 phenotypes. I was unable to

Figure 2.3 GOT phenotype frequencies in Meta, and assumed genotypes.



GOT phenotype	1	13	123	15	3	35	345	135	5	
Genotype	aa	ad	ab	ac	bb	be	cb	cf	cc	N
M.merianae	0.98	0.004	0.007	0.007	0.004	--	--	--	--	298
M.menardi	0.995	--	--	0.005	--	--	--	--	--	190
M.segmentata	0.004	--	0.004	--	0.98	0.004	0.004	--	0.004	233
M.mengei	--	--	--	0.019	0.004	--	0.067	0.004	0.907	569

Figure 2.4 LDH phenotype frequencies in Meta, and assumed genotypes.

origin $\overset{+}{-}$ ———————————————————————————

LDH phenotype	1	123	13	3	4	4	456	
Genotype	aa	ab	eb	bb	cc	dd	cf	N
M.merianae	0.008	0.119	--	0.87	--	--	--	243
M.mengei	--	--	0.003	0.995	--	--	--	383
M.menardi	--	--	--	--	--	1.0	--	206
M.segmentata	--	--	--	--	0.987	--	0.013	225

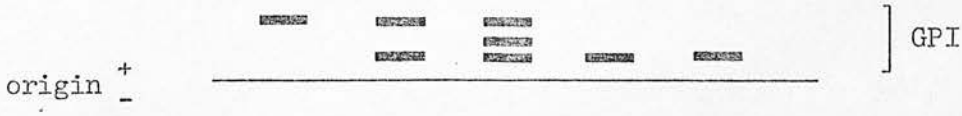
localise minor band activity to specific tissues (leg muscle, heart, haemolymph) and I assume that these represent LDH subunits which are split into two populations by *in vitro* binding with coenzyme. Because their minor band activities have different relative mobilities, therefore, *M. segmentata* LDH4 is an allozyme of *M. menardi* LDH4. The minor bands stained infrequently, but occasionally one or both occupied positions on the gel other that those shown in Figure 2.4. Given that these do in fact represent subunit activities, this result indicates hidden genetic variation within major band positions and I am not at all confident that the simple genotypic interpretations of LDH are accurate.

Spider LDH is quite unlike vertebrate LDH. The former is specific for the D-lactate isomer (Long and Kaplan 1968) and the latter is a tetramer as opposed to a dimer. Selander et. al. (1970) believe that *Limulus* LDH is controlled by two cistrons, but if this was the case in *Meta* one would not expect to find so much genetic variation within species at this "locus". Harris, Hopkinson, and Edwards (1977) argue that two-cistron enzymes are less polymorphic than single-locus multimeric enzymes, which in turn are less polymorphic than single-polypeptide enzymes.

The majority of PGI assays yielded deeply stained streaks rather than bands and after a while this assay was discontinued. PGI is a dimer and this locus is also differentiated and diagnostic for *M. menardi* (Figure 2.5), though not at the 0.01 level. Figure 2.5 also shows the relative band position of α-GPDH which was monomorphic.

In practice, one does not need formal estimates of proportions of overlap of genotype frequencies to use these loci as diagnostic characters. Indeed, their systematic value was intuitively obvious

Figure 2.5 PGI phenotype frequencies in Meta, and assumed genotypes.



GPI phenotype	1	13	123	3	3	
Genotype	aa	ac	ab	bb	cc	N
M.mengei	--	--	--	1.0	--	80
M.segmentata	--	--	0.018	0.982	--	57
M.merianae	0.059	--	0.118	0.795	--	34
M.menardi	0.92	0.074	--	--	0.008	122

because the great majority of specimens fell into one of four categories: LDH3, MDH3, GOT5, PGI3 (*M.mengei*); LDH4, MDH1, GOT3, PGI3 (*M. segmentata*); LDH3, MDH1, GOT1, PGI3 (*M. merianae*); LDH4, MDH2, GOT1, PGI1 (*M. menardi*). And in the event of a specimen being heterozygous at one locus, that locus could be overlooked and assignment to species made on the basis of the remaining loci.

No matter how accurate or otherwise is my interpretation of the genetics at these loci, the four common combinations of enzyme phenotypes speak for themselves. In my experience they are more reliable than are the few morphological features which distinguish adult female *M. mengei* from *M. segmentata*. The problem of unidentifiable juveniles is frequently encountered in population studies of spiders, and had it not been for the zymogram technique it would have been difficult to unravel the overlapping life histories of the Scottish *Meta* populations (Chapter 4). Gaining reinforcement from their allozymes, I did learn to recognise colouration differences between the first two post-eggsac instars of these siblings, but from that stage onwards their colours converge and no morphological character is particularly reliable, except in adult males. Once the colour and shape differences between *M. merianae*, *M. menardi*, and the sibling pair are noticed, juvenile *M. merianae* and *M. menardi* are quite distinctive, although it was surprising to find that juvenile and adult *M. menardi* are coloured so differently (Figure 1.1). I associated the two forms on the basis of their enzyme phenotypes before confiming this with laboratory cultures.

The value of biochemical characters remains largely unrealised in invertebrate systematics outside *Drosophila* research (Wagner and Selander 1973) and as far as I know noone has applied biochemical genetics to spider systematics before. The small sample of loci

used here may also prove useful in separating other pairs of sibling spider species. Certainly, in a cursory experiment using 2-5 spiders per species they appeared to be differentiated among the genera Meta, Tetragnatha, Araneus, Zygiella, Theridion, Linyphia, and Pardosa. If a larger sample of loci were studied in detail this approach could be used effectively to resolve some of the current uncertainties in spider taxonomy. One specific example of this which I know of is the questionable taxonomic status of North West / South East European Araneus cornutus which Thaler (1974) reports should be revised into two species. This is the kind of problem for which the simple electrophoresis methods which I used are invaluable.

For the present study I erred on the side of simplicity. Having found a sample of four loci which, when used in combinations of two or three, provide unambiguous positive species identification without the need for progeny studies, I simply used the technique as part of routine sampling and analysis. I did not study the genetics at these loci per se because this would neccessarily have involved optimising the resolution and separation of allozymes and characterising them by systematically comparing results on a number of gel systems at various pH's; checking the homogeneity of alleles with other methods - isoelectric focusing for example; and consolidating the conclusions with progeny studies. The rewards which come with approaching electrophoresis systematically are well illustrated by Coyne (1976) and Singh, Lewontin, and Felton (1976). These authors analysed homogenates from monogenic lines of Drosophila pseudoobscura and D. persimilis with two acrylamide gel concentrations, at two pH's, and with various degrees of heat denaturation treatment of the enzymes. Not only did they find that each "allele" at the xanthine dehydrogenase locus could be split into several treatment-defined allozymes,

but also that these two sibling species have much less overlapping gene pools than had previously been recognised (Ayala and Powell 1972). Interestingly, their results showed also that the Bogotá D. pseudoobscura population, which had been thought to be a recently established founder population in the process of speciation, is of ancient origin and has a number of unique alleles at the XDH locus.

Reliable measures of degrees of overlap between populations belonging to different taxa, or average allelic identities (Nei 1972), are of great value in quantifying the degrees to which species have diverged. The loci illustrated in Figures 2.2 - 2.5 cannot be looked upon a a random sample of the spiders' genomes because two esterase loci and one MDH locus were not scored routinely and the data are biased towards the most differentiated loci. It would, therefore, be inappropriate to comment on taxonomic distances between these species in absolute terms. Nevertheless, pairwise comparisons of normalised allelic identities (Nei 1972; Webster and Burns 1973) are instructive. Essentially, these are correlation coefficients computed from the frequencies of all the alleles (within each species) at each locus and the frequencies of the alleles shared by the species-pair. Terms are averaged over loci. The normalised identities in Table 2.3 were computed with gene frequencies estimated from the phenotype frequencies in Figures 2.2 - 2.5 assuming Hardy-Weinberg equilibrium. Two values are given for M. mengei - M. merianae: the upper figure takes Figure 2.3 at face value, and the lower figure assumes that these species share only the LDH^a allele and that M. mengei LDH3 is an allozyme of M. merianae LDH3. Evidence for this is given in Chapter 3.

The identity of the M. segmentata and M. merianae MDH1 phenotype is the main contributor to the close similarity between these

Table 2.3 Pairwise normalised identities of alleles at the α-GPDH, MDH, LDH, GOT, and PGI loci.

	M.segmentata	M.merianae	M.menardi
M.mengei	0.4224	0.589 (0.404)	0.213
M.segmentata		0.6884	0.2061
M.merianae			0.4444

The allelic identity of populations X and Y with respect to the jth locus is: $I_j = j_{xy} / (j_x j_y)^{\frac{1}{2}}$; where $j_x = \sum x_i^2$, $j_y = \sum y_i^2$, and $j_{xy} = \sum x_i y_i$. x_i and y_i are the frequencies of the ith allele in the two populations.

The average allelic identity of populations X and Y over several loci is: $I = J_{xy} / (J_x J_y)^{\frac{1}{2}}$; where J terms are the arithmetic means of the j terms at all loci. (Nei 1972).

species and had the two esterase loci been taken into account, M. mengei and M. segmentata would have been the most alike of the pairs. The later are more like M. merianae than M. menardi, and M. merianae is almost as like M. menardi as it is to the morphological siblings. If the overlap at the two esterase loci is taken into acount, the four species can be ranked along an axis of genetic similarity in the order: M. mengei, M. segmentata, M. merianae, M. menardi; though the positions of M. mengei and M. segmentata may in reality be the reverse, as they are when the species are ordered according to ecological similarities (Figure 4.8).

The conditional tenses in the last paragraph illustrate the limitations of the method when it is applied to a quantitative problem. Although it would be impractical to establish monogenic lines of Meta species, there is scope for improvement. As it happened, starch gel electrophoresis served its function as a method of species identification, but a study which focused on genetic distances would, I am sure, prove fruitful. Spider systematics would yield to a fresh approach, and at least one problem - the phylogenetic position of Meta in relation to the Araneidae and the Tetragnathidae - is waiting to be solved.

Chapter 3. Biochemical Adaptation.

It is not unusual for population studies of spider-sized invertebrates to involve genetic dissection of samples of 10 - 20 enzyme loci, but of the 17 enzymes assayed in Meta by the zymogram technique only non-specific esterases, α-GPDH, LDH, MDH, GOT, and PGI were sufficiently active to be scored reliably. The insensitivity of the starch gel method may partly account for this result, but it also reflects the metabolic adaptations of spiders in general.

Linzen and Gallowitz (1974) demonstrated that the enzyme profile of Cupiennius salei (Lycosidae) is heavily weighted in favour of anaerobic glycolysis and that muscle tissues are virtually devoid of mitochondria. Meta leg and heart muscles have no detectable mitochondria on electron micrographs either (J. E. Hillerton, pers. com.). Linzen and Gallowitz proposed that GTP (glutamate-oxaloacetate transaminase), GOT, and MDH are at the centre of cytoplasmic NAD^+ regeneration during muscle activity thus:

$$\text{pyruvate} + \text{glutamate} \xrightleftharpoons{\text{GPT}} \text{alanine} + \alpha\text{-ketoglutarate}$$

$$\alpha\text{-ketoglutarate} + \text{aspartate} \xrightleftharpoons{\text{GOT}} \text{glutamate} + \text{oxaloacetate}$$

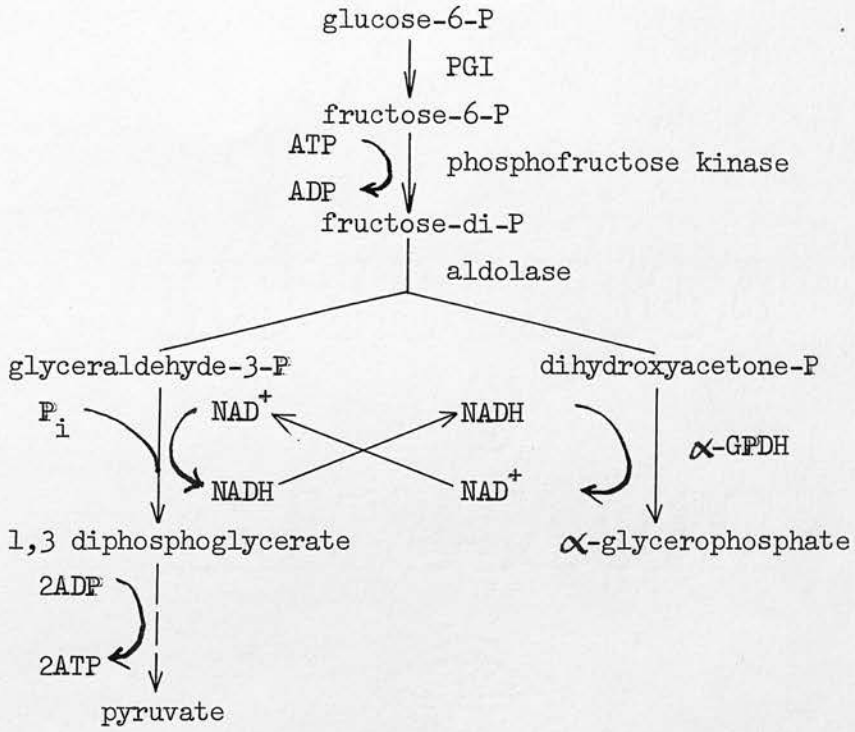
$$\text{oxaloacetate} + \text{NADH} \xrightleftharpoons{\text{MDH}} \text{malate} + NAD^+$$

$$\text{pyruvate} + \text{NADH} + \text{aspartate} \rightleftharpoons \text{alanine} + \text{malate} + NAD^+$$

In their experiments LDH was also very active and this regenerates NAD^+ too:

$$\text{pyruvate} + \text{NADH} \xrightleftharpoons{\text{LDH}} \text{lactate} + NAD^+$$

Linzen and Gallowitz did not assay ∝-GPDH or PGI activities, but during anoxia in insect flight muscle (which has no LDH activity) these enzymes are involved in the following scheme (Hochochka and Somero 1973):



Thus malate, lactate and ∝-glycerophosphate may all act as terminal H^+ acceptors in place of oxygen during anaerobic metabolism, but the three alternative NAD^+ regenerating pathways are probably integrated by substrate concentrations in such a way that together they result in a graded response to hypoxia, which in turn depends on the "urgency" and duration of the spiders' energy requirements. During normal glycolysis all of the carbon atoms pass along the left hand arm of the scheme above. Assuming that glycogen is the fuel, which is probably the case in spiders (Linzen and Gallowitz 1974), involvement of LDH for the removal of pyruvate and NAD^+ regeneration yields three moles of ATP per mole of glycogen consumed, and per 2 moles of pyruvate produced. However, the LDH reaction leads to a metabolic

cul-de-sac and all of the lactate must be reconverted to pyruvate for degradation in the TCA cycle. Back-pressure from accumulated lactate limits the scope for anaerobic metabolism using LDH. When the GPT-GOT-MDH pathway is coupled, on the other hand, pyruvate is permanently removed from the system and the malate so formed can enter the TCA cycle later, being converted to oxaloacetate in the mitochondria by the cathodal MDH isozyme (Figure 2.2). The normal entry of the carbon skeleton into the TCA cycle via pyruvate and acetyl CoA involves a NADH yield, but with this system NAD^+ is regenerated, while at the same time the allosteric effect of pyruvate on enzymes earlier on in glycolysis is minimal. When α-GPDH converts dihydroxyacetone phosphate to α-glycerophosphate in the cytoplasm, only one mole of ATP is produced per mole of glycogen consumed. This is inefficient, but because the low ATP yield is fuelled from behind, the reaction is independent of pyruvate formation and a "quick start" to glycolysis is ensured. In insects, the α-glycerophosphate formed in this reaction scheme crosses the mitochondrial membrane, is oxidised to dihydroxyacetone phosphate by a mitochondrial α-GPDH isozyme, and returns to the cytoplasm where it is once again reduced by the α-GPDH reaction. Thus α-GPDH is involved in shuttling electrons and protons across the mitochondrial membrane; but since only one α-GPDH isozyme was detected on zymograms of Meta enzymes, α-glycerophosphate may act as a temporary proton sink in the same way as lactate does.

These conclusions conform with other aspects of spider physiology. The resting metabolisms of spiders are lower than those of other spider-sized ectotherms (Anderson 1974) and tissues are supplied mainly by dissolved oxygen (Angersbach 1975: Eurypelma helluo; Mygalomorphae). Spider haemocyanin has an unusually high O_2

affinity and venous haemolymph is 90% saturated at rest, but when pO_2 and pH fall from resting levels significant unloading of haemocyanin-bound oxygen occurs (Loewe and Linzen 1975: Cupiennius). The physiological function of spider haemocyanin, like vertebrate myoglobin, is to store oxygen during rest and release it during activity, and not primarily to transport O_2 to respiring tissues, which is the function of haemoglobin in vertebrates. Furthermore, O_2 diffusion paths are long and O_2 gradients are shallow, so rapid muscular contraction must rely on ATP supplied by anaerobic glycolysis: O_2 is reduced mainly after bouts of activity, during rest and recovery.

Animal foraging strategies can be ordered along a continuum between "time-efficiency", where energy is expended maximising the food yield in a unit of time, and "energy-efficiency", where time is spent and energy is conserved waiting for food items to come within striking distance (Enders 1975). Although hunting manners differ among spider families, no spider expends much energy on continuous locomotion, and all spider foraging strategies depend to a greater or lesser degree for their success on sudden bursts of activity followed by relatively long periods of quiescence. In fact, spiders are generally incapable of sustaining prolonged activities such as running (Bristowe 1941; Surtees 1976).

The discussion above makes the point that the starch gel electrophoresis method (pages 8 and 9 as opposed to more rigourous methodology; page 16) biases samples of enzyme loci towards those enzyme systems which enable an organism to stay at the front line of the competition for resources and reproductive advantage. But it sheds no light on the question of whether these particular metabolic enzymes (esterases are not at the front line of ATP turnover even though they are active on zymograms) are prone to rapid evolution and

genetic differentiation during any of the three principle stages of speciation and ecological divergence (Lewontin 1974).

There is some evidence that, on average, variable-substrate enzymes such as esterases are more polymorphic than glucose metabolism enzymes (Kojima, Gillespie, and Tobari 1970), and that enzymes which occupy regulatory positions in metabolic pathways are more polymorphic than non-regulatory enzymes (Johnson 1974). Of the six metabolic enzymes, only PGI falls in Johnson's regulatory enzyme category. The total sample of loci in the present data is much too small and unrepresentative to comment further on the statistical relationship between polymorphism per se and enzyme function; or whether some enzymes are polymorphic because their surfaces contain many "neutral" sites (Harris, Hopkinson, and Edwards 1977) and others because over-dominance maintains several allozymes in populations (balanced polymorphism). I felt that a close look at functional differences (as opposed to electrophoretic differences) between Meta allozymes at the LDH, MDH, or GOT loci might at least point to an answer to this question.

One hypothesis seemed promising. The ecology of each of the four Scottish Meta populations is unique in some aspect of the multidimensional niche and interspecific differences of breeding season, diel cycle, and microhabitat probably set up differences in the temperature regimes the spider species experience. Enzymes lower the free energies of activation of chemical processes so that they may proceed at physiological temperatures. Temperature variations of a few degrees can have potentially profound effects on enzyme catalysed reactions in ectotherms by altering the proportion of intermolecular collisions which have sufficient kinetic energy to reach activation. However, by influencing the stabilities of weak

chemical bonds, temperature variations can also modulate the kinetic properties of enzymes. Many ectotherm enzymes have been shown to respond to temperature changes in such a way that they maintain their regulatory functions over habitat temperature ranges, and allozyme and isozyme differences between conspecific and heterospecific populations can often be related to the native habitat temperatures of the populations (eg. Koehn 1969; Hochachka and Somero 1968; Somero 1969; Merritt 1972). Hochachka and Somero (1973) review the subject of temperature compensation at the enzyme level in ectotherms.

According to the Michaelis-Menton model, enzyme catalysis is essentially a three-step process:

$$E + S \underset{k-1}{\overset{k+1}{\rightleftharpoons}} ES \rightleftharpoons ES^{*} \underset{k-2}{\overset{k+2}{\rightleftharpoons}} E + P$$

(Dixon and Webb 1964)

where E, S, and P are the enzyme, substrate, and products; ES is the enzyme-substrate complex, and ES^{*} is the activated complex; and k+1, k-1, k+2, k-2 are rate constants. The effect of temperature on the concentration of ES^{*} can be observed experimentally by its effect on k+2 under substrate saturation conditions; ie. when all of the enzyme is bound to substrate. Vmax, the initial reaction rate at the substrate saturation concentration (ie. before there is significant back-reaction due to the build up of products), estimates k+2, and the relationship between Vmax and temperature can be used to measure enthalpy of activation. $V_{max} = [E]\, k+2$ under these conditions.

There is some evidence that enzymes in cold-adapted populations have lower acivation energies (Ea's) than enzymes in warm-adapted populations (Vroman and Brown 1963; McNaughton 1972, 1974), but because physiological substrate concentrations may never reach

saturation, Ea may be of secondary importance in the evolution of automatic rate compensation at the enzyme level (Somero 1969). Instead, at physiological substrate concentrations catalytic rate is sensitive to enzyme-substrate affinity modulation. Enzyme-substrate affinity is inversely proportional to the Michaelis constant (Km), which is an experimental estimate of the substrate equilibrium constant (Ks). $Ks = [ES] / [E][S] = k-1 / k+1$. Km equals the substrate concentration required to half-saturate the enzyme.

Natural selection alters the Km of an enzyme to within one order of magnitude of its physiological substrate concentration; in this situation catalytic rate is most sensitive to substrate concentration changes and to modulation of Km (Cornish-Bowden 1976). Enzymes evolve sensitivity to modulators in order to maintain their regulatory functions, rather than to maximise catalytic rate per se (Somero 1969). Ectotherm enzymes generally exhibit positive thermal modulation of Km (ie. an inverse relationship between temperature and enzyme-substrate affinity) over the habitat temperature range, the adaptive value of which is that there is in-built thermal independence in biochemical pathways. The range of positive thermal modulation is, however, limited by the enzymes' regulatory roles, which necessitate a close correspondence between Km and substrate concentration (Hochachka and Somero 1973). The following experiments were designed to investigate interspecific differences in thermal modulation of Km in Meta.

Methods.

This part of the research was undertaken in collaboration with Susan Auld (Auld 1976). I was responsible for design and methods, determination of Km values of the LDH reactions of the four species, and for investigating thermal modulation of M. segmentata MDH $Km_{oxaloacetate}$. Auld examined thermal modulation of Km's in the forward direction for M. mengei, M. segmentata, and M. merianae LDH.

The methods used were essentially straightforward enzymological techniques, using the rate of change of NADH absorbance at 340nm to assay initial reaction velocities under various conditions of enzyme concentration, substrate concentration, pH, and temperature. However, because of the time factor, it was necessary to compromise the design by using centrifuged crude homogenates of whole spiders as the source of enzyme. Ideally, homogenates of monogenic lines should have been used as allozyme sources, but in this case that was impractical. On the advice of Dr. C. H. Sissons, purification of the LDH (and MDH) fractions was considered unnecessary.

Frozen spiders were identified (only adult males of M. mengei and M. segmentata were used), homogenised in 0.1M phosphate pH 8.0, centrifuged at 14,000g for 15 minutes, diluted to approximately 1 : 50 (wt : vol), and stored in the deep freeze. It was assumed that in samples of 20 adult spiders of each species the proportion of the wild type allozyme would be high and that the results would be determined mainly by the properties of the allozyme in proponderance. The purity of each homogenate was tested on starch gels, but rare allozymes, being dilute, were not detected, even though they may have been present. In the case of MDH, anodal and cathodal isozymes freely mix in homogenates and the assays were regarded as measures of the activity of the "average genotype" in the M. segmentata population.

Reaction velocities were measured at constant temperature and pH with a Unicam SP800 UV spectrophotometer and SP21 slave recorder at 340nm. Reaction temperatures were maintained by water circulating through the reaction cell holders and an external water bath. A cooling unit was used against the heating element of the water bath at temperatures below 20°C.

0.1M Na_2HPO_4 and NaH_2PO_4 were titrated at experimental temperatures with a pH meter, and substrates were weighed to the nearest 0.1mg and dissolved in 50ml buffer. NADH concentrations were measured by optical density at 340nm and all concentrations are expressed in mM, except lactate which is expressed in g Na D-L lactic acid syrup (Sigma) ml^{-1}. Stocks of substrates were made up with micropipettes before each experiment. LDH reaction volumes consisted of 400µl constant substrate, 500µl varying substrate, and 100µl enzyme. MDH reaction volumes were made up of 500µl of each substrate and 50µl of enzyme. 1.3ml Spectrosil cuvettes were used.

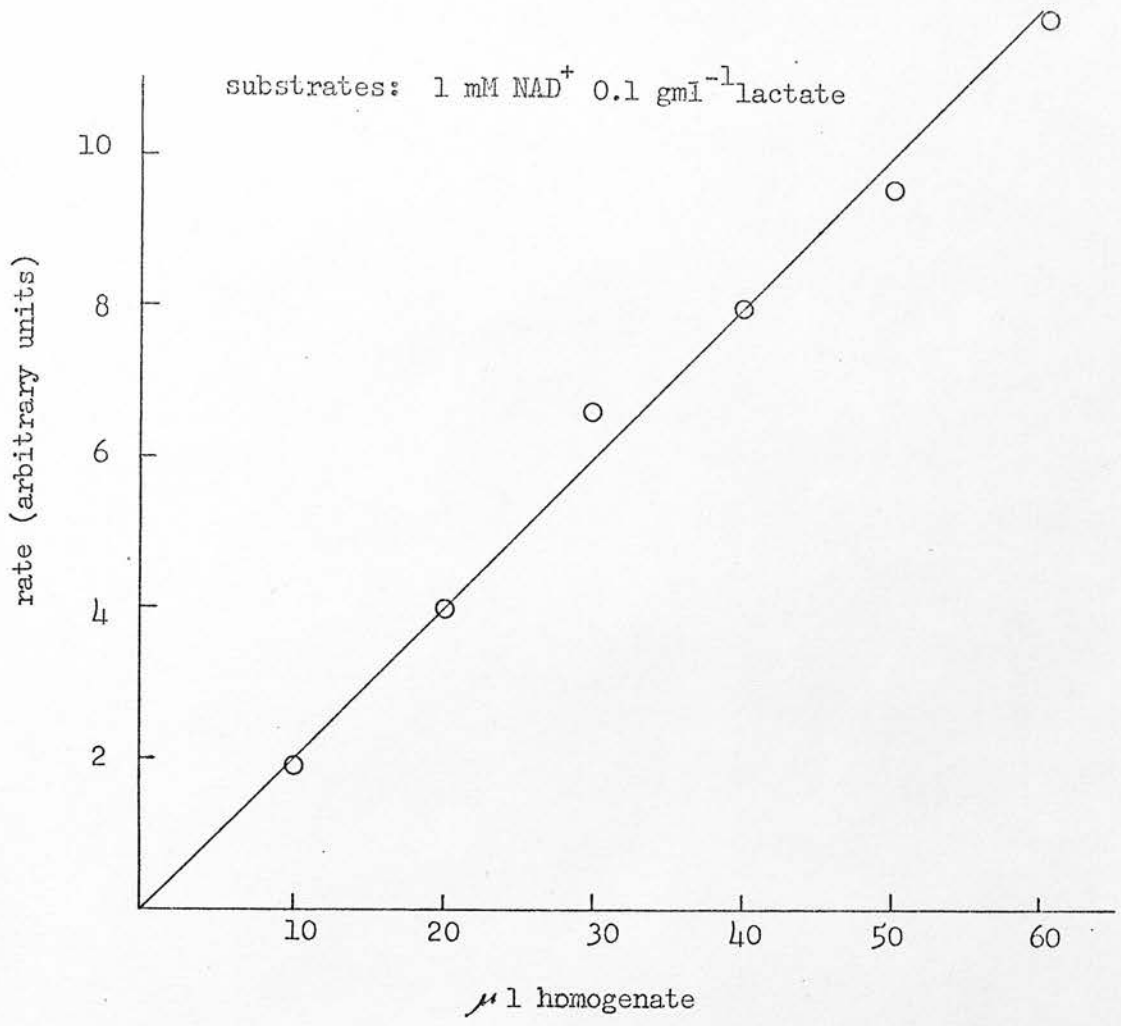
Substrates were allowed to equilibrate with the experimental temperature for several minutes in the spectrophotometer cell holders. Reactions were begun by adding the enzyme and inverting the cell once to mix its contents. Two to four assays were made at each experimental condition and initial velocities were obtained by the method of Henderson (1971). Mean rates were used in graphical analyses, which appear in Appendix 2.

Results and Discussion.

The first experiment in this series was a test for the presence of competitive LDH inhibitors in crude homogenates. Figure 3.1 illustrates concentration curves for M. mengei LDH for the forward and back reactions at 25°C pH 8.0. The lines are at 45° which indicates that reaction velocity is directly proportional to enzyme concentration and that no inhibitors were present in the homogenate, the concentration of which would also have doubled with each doubling of enzyme concentration. The lower graph shows that in the presence of 200µg rabbit LDH increasing concentrations of spider LDH activity are not additive: rabbit LDH inhibits spider LDH. The reason for this is not clear, but it is possible that because the enzymes are specific for different lactate isomers (Long and Kaplan 1968) their active sites interfere. Both lactate isomers were present in the substrate of all lactate experiments.

Table 3.1 reports Km's for each LDH substrate at 25°C pH 8.0. This pH was used for consistency with electrophoresis assays, and the experiment was aimed at comparing the properties of allozymes which occupy similar positions on zymograms (Figure 2.4). Table 3.1 shows that M. segmentata and M. menardi LDH4's are distinct allozymes. M. mengei and M. merianae LDH3's, however, differ markedly only in Km_{NAD^+}. Unfortunately, the M. mengei Km_{NAD^+} was the least accurate of the determinations (Appendix 2: points are scattered), but these allozymes were consistently different in Auld's experiments. While screening one batch of M. merianae LDH against M. mengei LDH on zymograms, the two band positions of their LDH3's differed slightly and I conclude that some aspect of the treatment of homogenates had

Figure 3.1 M. mengei LDH enzyme concentration curves at 25°C.



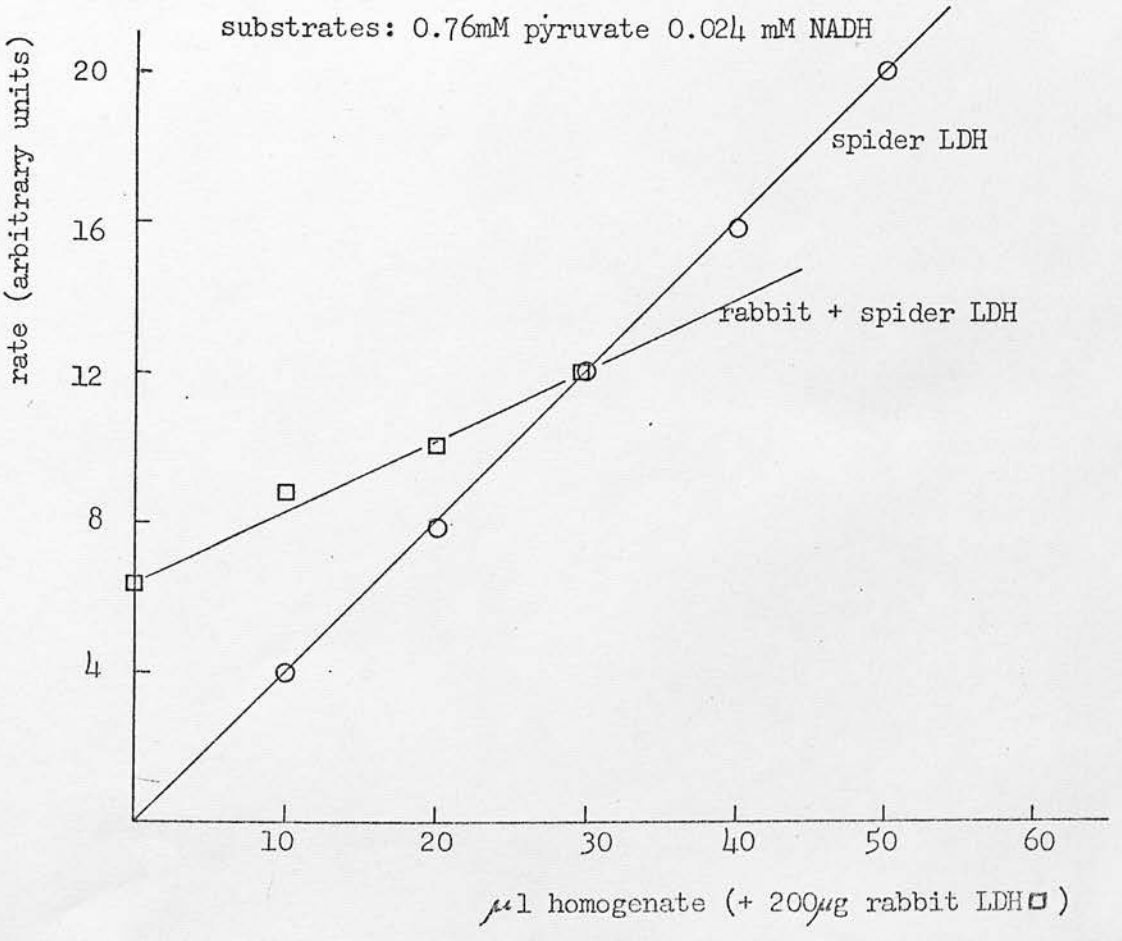
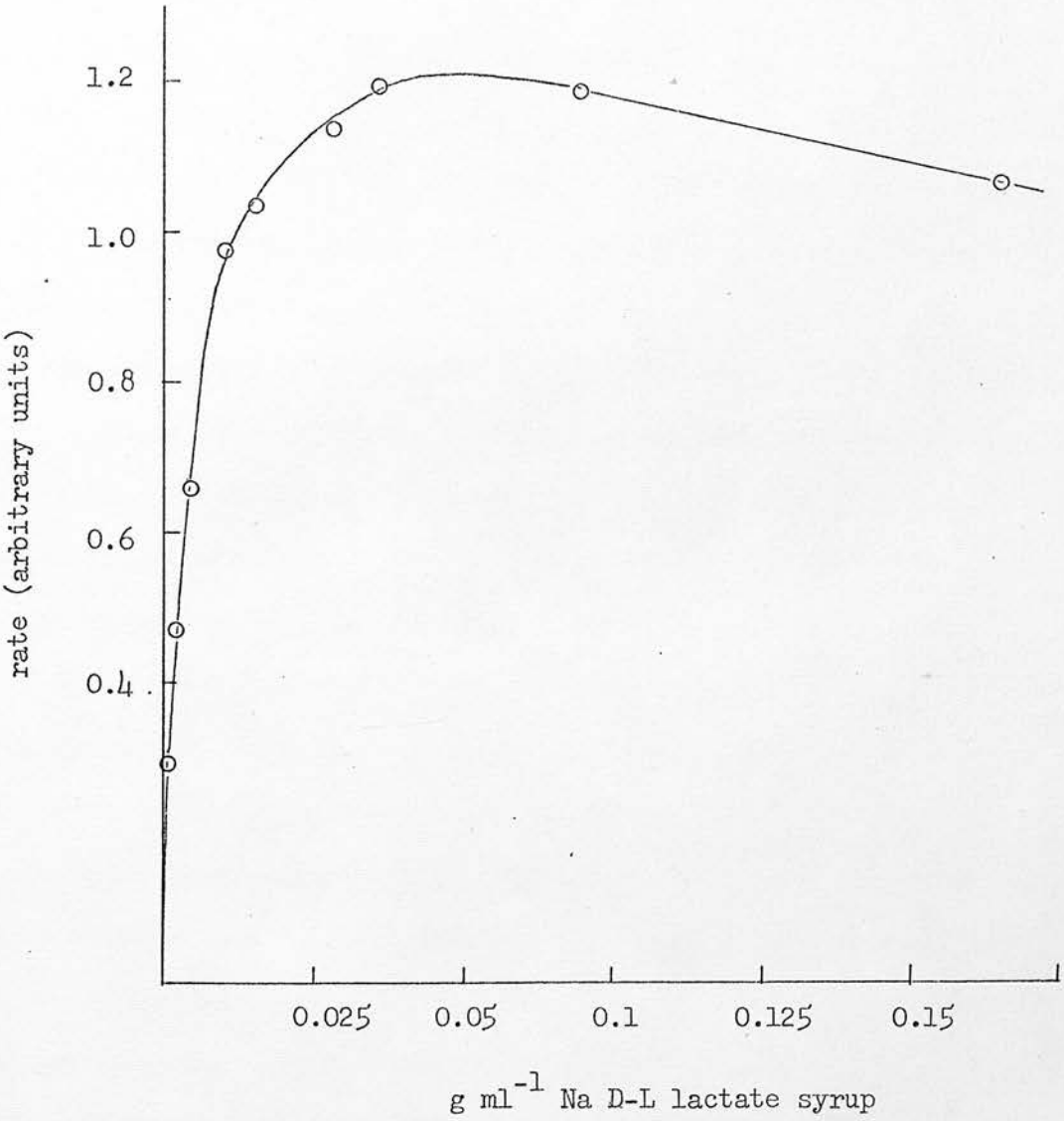


Table 3.1 Meta LDH Km's at 25°C pH 8.0.

	$Km_{lactate}$	Km_{NAD^+}	$Km_{pyruvate}$	Km_{NADH}
M. mengei	0.009	0.087	0.065	0.0096
M. merianae	0.007	0.115	0.059	0.01
M. segmentata	0.07	0.161	0.141	0.14
M. menardi	0.021	0.076	0.048	0.018

Km_{NAD^+}, $Km_{pyruvate}$, and Km_{NADH} are in mM, and $Km_{lactate}$ is in g ml^{-1} Na D-L lactic acid syrup.

Figure 3.2 Enzyme saturation curve of M. menardi LDH showing substrate inhibition by lactate at 25°C.



altered the charges of the LDH allozymes sufficiently for them to separate on starch gels. However, the result was not repeatable.

These experiments showed also that spider LDH exhibits substrate inhibition by lactate at high lactate concentrations. Figure 3.2 illustrates an example of this. Although the effect is not pronounced, it shows that the enzyme behaves as a leaky one-way valve at high lactate concentrations, slowing down the reconversion of NAD^+ to NADH. This is significant because it illustrates the point made on page 21, that the scope for anaerobic metabolism using the LDH reaction is limited by back-pressure from accumulated lactate. This adaptation of the enzyme aleviates that problem to some extent. No substrate inhibition by pyruvate was detected.

Auld (1976) showed that in the forward direction spider LDH has a plateau of activity between pH 5.5 and pH6.5. The enzyme denatures below pH 5.0. pH 6.5 was used for temperature versus Km experiments, the results of which are illustrated in Figures 3.3 and 3.4. These experiments were designed to detect interspecific differences in the ranges over which positive thermal modulation operates. The M. segmentata allozyme was expected to exhibit the most "warm adapted" response to temperature, with the Km modulation curve shifted over towards higher temperatures than the allozymes of the other two species. The reason for this is that M. segmentata forages only during the summer months and does not overwinter. The M. merianae curve was expected to be shifted over towards low temperatures compared with the allozymes of the other species. M. merianae and M. mengei overwinter twice in Scotland before maturing and M. merianae lives only in shaded places. Adult M. merianae are nocturnal whereas M. mengei and M. segmentata are diurnal.

Figure 3.3 shows that for $Km_{pyruvate}$ and Km_{NADH} there is no

Figure 3.3 Thermal modulation of $Km_{pyruvate}$ and Km_{NADH} (from Auld 1976)

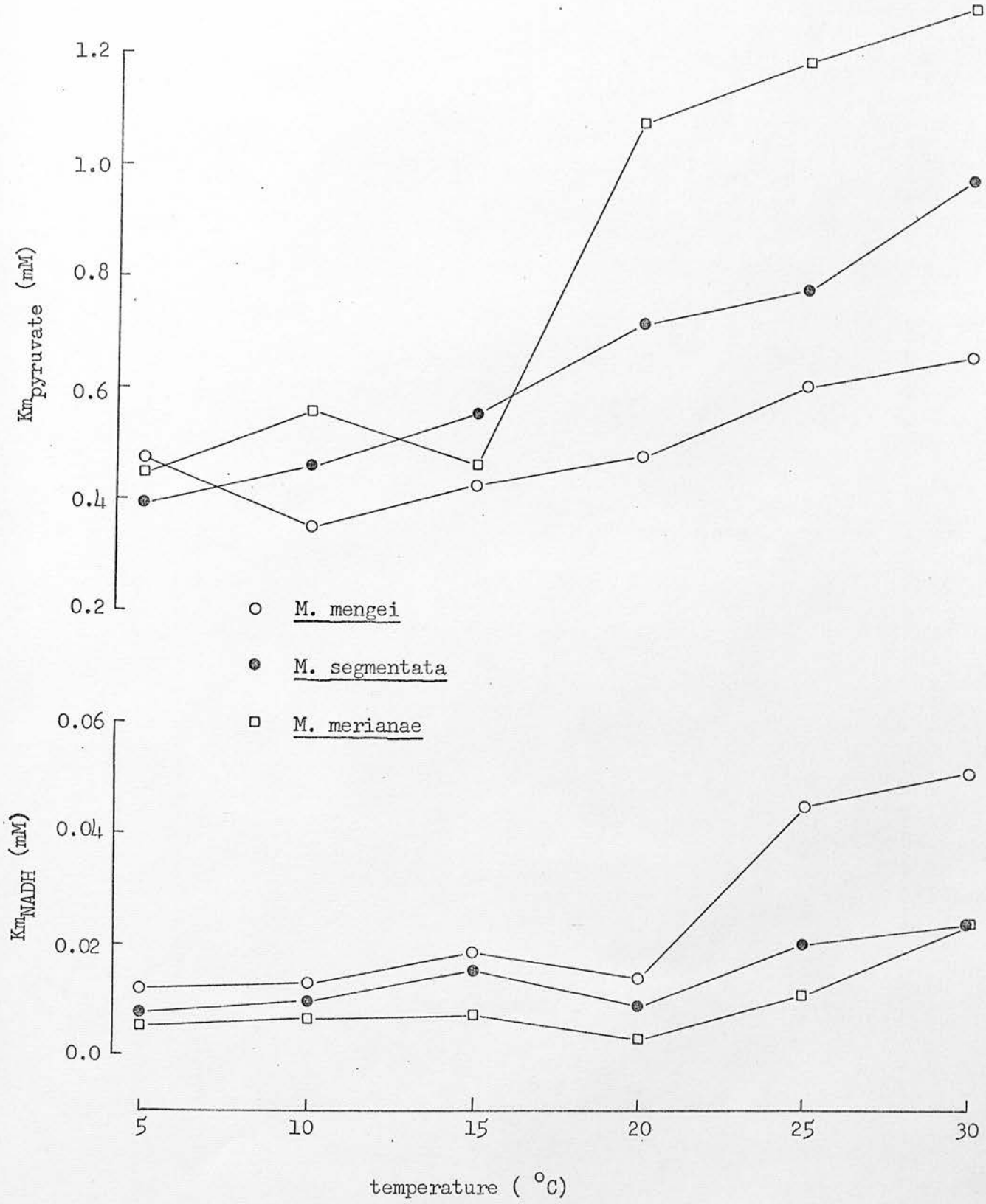


Figure 3.4 $Vmax_{pyruvate}$ and $Vmax_{NADH}$ versus temperature (from Auld 1976)

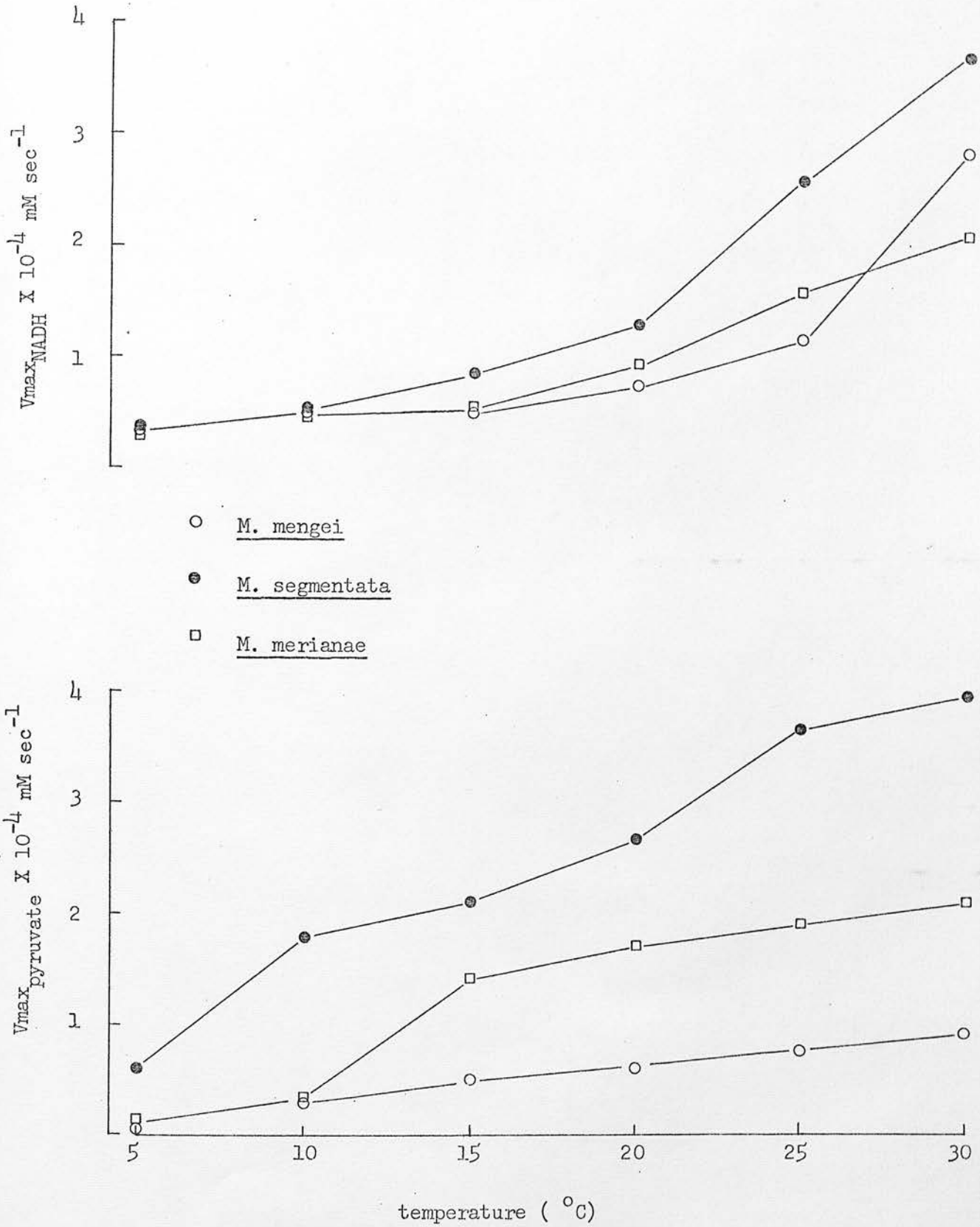


Table 3.2 LDH activation energies (Kcal $mole^{-1}$).

	Ea pyruvate		Ea NADH	
	5-15°C	15-25°C	5-15°C	15-25°C
M. mengei	34.51	7.0	5.35	15.06
M. segmentata	17.57	9.69	12.57	21.22
M. merianae	80.42	5.15	9.45	19.1

Activation energies were obtained from the formula

$Ea = RT_1T_2 \ln Q_{10} \; / \; 10^{\circ}K$; where R = 1.986 cal $^{\circ}K^{-1}mole^{-1}$; T_1 and T_2 are assay temperatures ($^{\circ}K$); Q_{10} was obtained from regression of Vmax on temperature, and equals the average Vmax change over the stated temperature ranges. (Dixon and Webb 1964)

obvious difference in the temperature ranges over which the positive thermal modulation of Km operates, but assuming that experimental error accounts for the low value for M. merianae $Km_{pyruvate}$ at 15°C, and also the apparent negative thermal modulation of M. mengei $Km_{pyruvate}$ between 5 and 10°C, the data show that the gradients rather than the ranges of $Km_{pyruvate}$ curves differ among the species, M. merianae having the steepest curve and M. mengei having the shallowest. None of the Km_{NADH} curves shows much response to temperature below 20°C, but above this temperature the M. mengei curve is steeper than the other two. The fact that $Km_{pyruvate}$ is more responsive to temperature variations than Km_{NADH} is, suggests that LDH activity is more closely linked to pyruvate concentration than to NADH concentration, a predictable result because pyruvate formation depends strictly upon glycolysis whereas NADH concentration may fluctuate due to the activities of many dehydrogenases.

Table 3.2 reports activation energies of the LDH reactions calculated from the data in Figure 3.4. These data show that Meta LDH exhibits positive thermal modulation of Ea_{NADH} and negative thermal modulation of $Ea_{pyruvate}$. Thus as the kinetic energies of enzyme-NADH collisions decrease, so does the barrier to activation. The reverse is true in the case of $Ea_{pyruvate}$, and the forward reaction becomes progressively thermodynamically unfavourable at low temperatures.

Taking Figure 3.3 and Table 3.2 together, the following conclusions can be drawn. The data show only slight positive thermal modulation of Km_{NADH} and positive thermal modulation of Ea_{NADH}. This suggests that Meta LDH has evolved to oxidise NADH at the maximum possible rate, an objective which is favoured by low Km_{NADH} and rapid

saturation of the enzyme. In this situation, Km is much less important than Ea in determining catalytic rate, and thermal modulation of Ea_{NADH} has evolved accordingly. The opposite is the case for pyruvate metabolism. $Km_{pyruvate}$ is at least four times higher than Km_{NADH} and the enzyme exhibits positive thermal modulation of $Km_{pyruvate}$, indicating that pyruvate concentration regulates LDH activity. The enzyme shows negative thermal modulation of $Ea_{pyruvate}$, but this may have little effect on catalytic rate because pyruvate concentration never reaches saturation. No interpretable pattern of interspecific differences emerges from these data.

The study of MDH $Km_{oxaloacetate}$ was limited to one species (M. segmentata) because had interspecific differences in this parameter been found it would not necessarily have followed that the anodal MDH phenotypes used in species identification (Figure 2.2) were responsible. Nevertheless, the experiments demonstrated that positive thermal modulation is not confined to LDH.

Figure 3.5 shows that MDH activity in the forward direction increases linearly with enzyme concentration, indicating that no inhibitor was present in the homogenate. Figure 3.6 shows that reaction velocity in the forward direction reaches a peak at about pH 7.75. 0.2M Tris-HCl buffer was used at pH 8.0 and above because phosphate looses its buffering capacity in this region. 0.1M phosphate was used at pH 7.75 for determinations of Km and Vmax.

Figure 3.7 illustrates positive thermal modulation of $Km_{oxaloacetate}$ for M. segmentata MDH and Figure 3.8 shows the dependence of Vmax on assay temperature. Repeat assays at 20 and 25°C illustrate the magnitude of experimental errors. The precise shape of the Km curve is debatable (whether it contains plateaux), but the trend is obvious and the magnitude of the change over the temperature range

Figure 3.5 M. segmentata MDH concentration curve at 25°C.

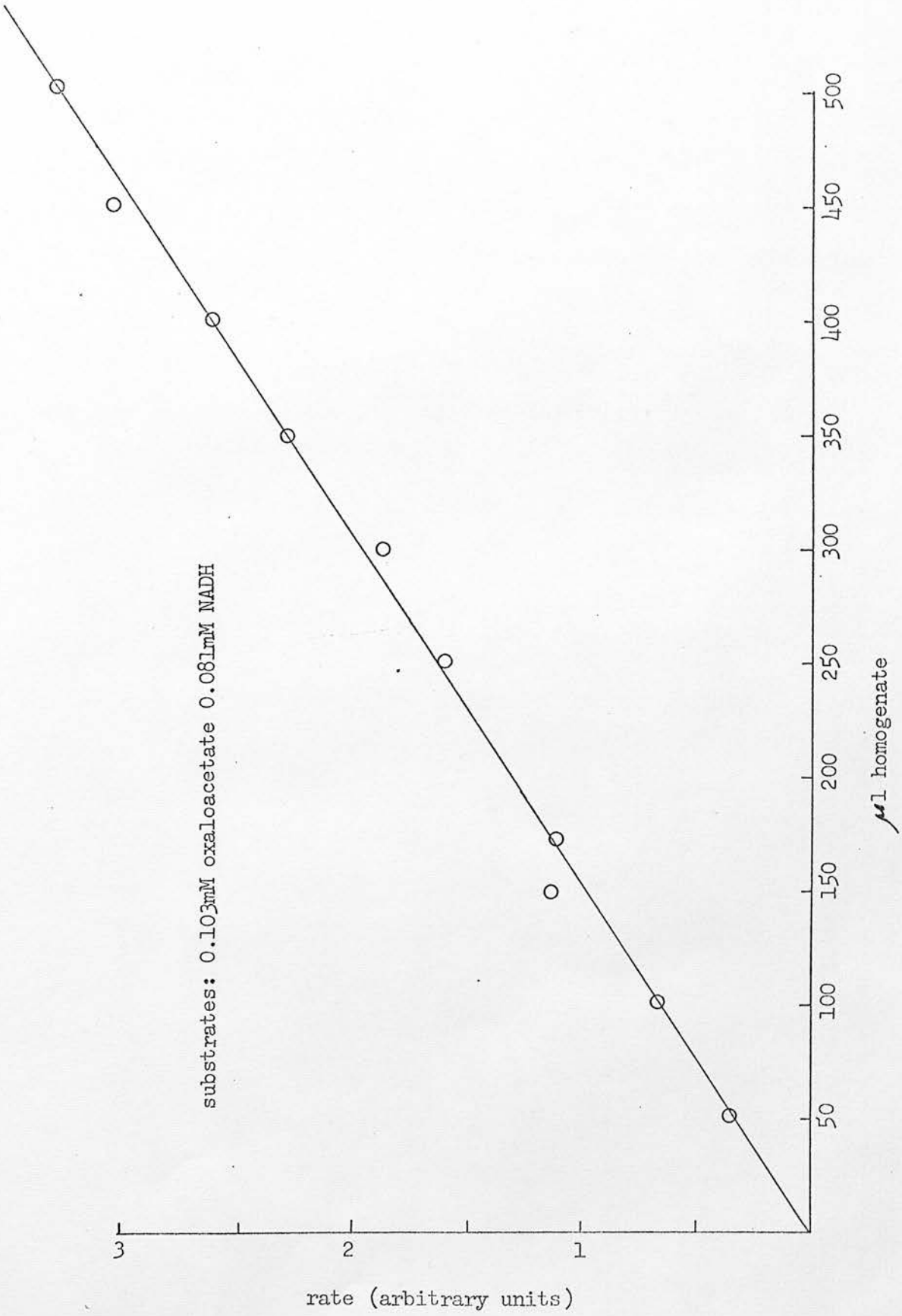


Figure 3.6 M. segmentata MDH pH curve at 25°C.

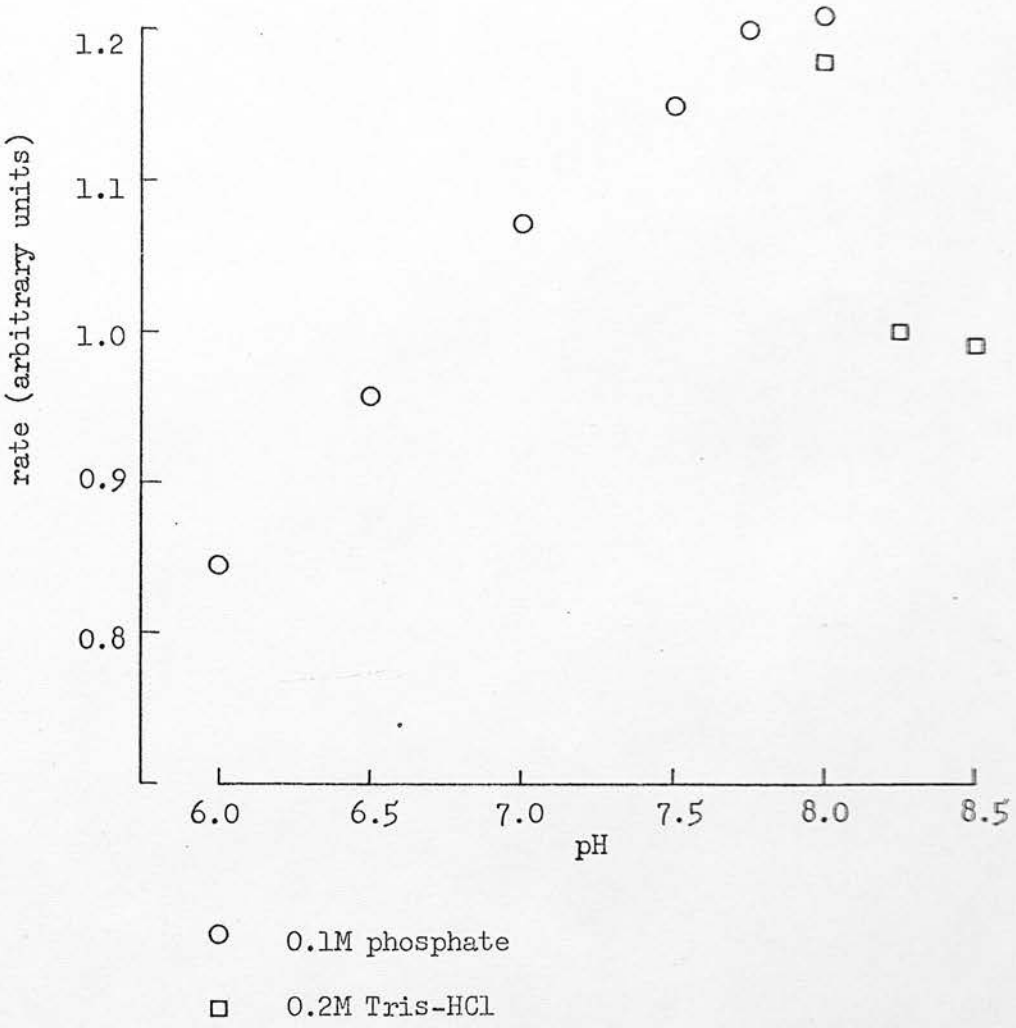


Figure 3.7 M.segmentata MDH $Km_{oxaloacetate}$ versus temperature.

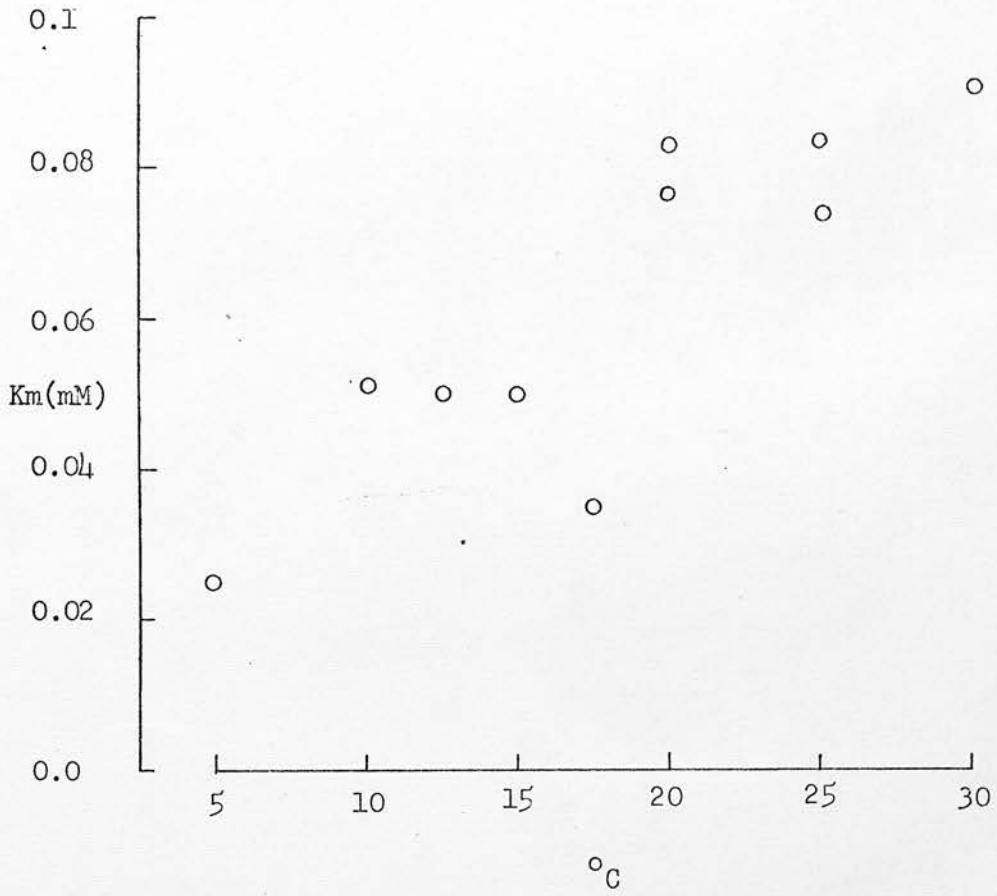
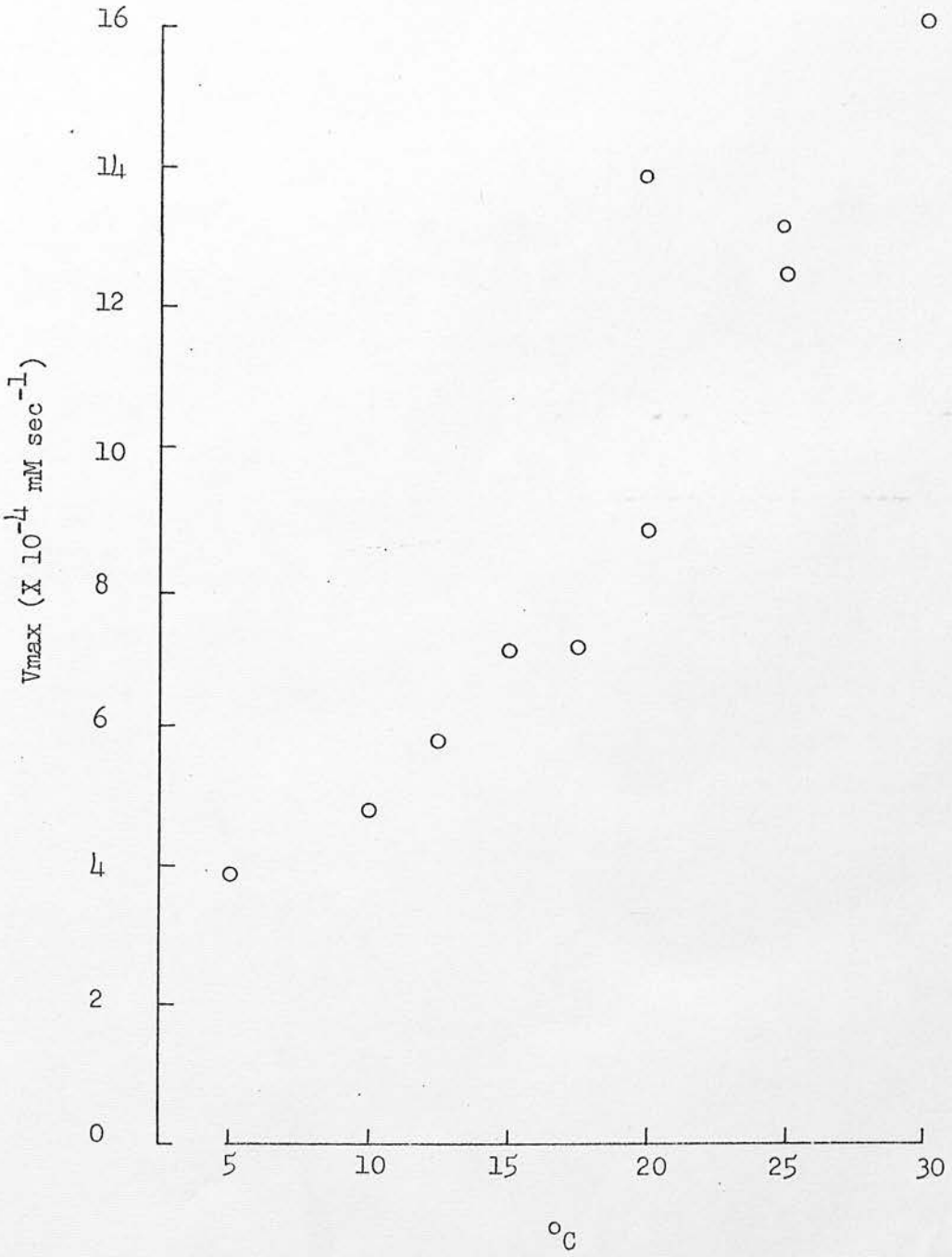


Figure 3.8 M.segmentata MDH V_{max} $_{oxaloacetate}$ versus temperature.



from 5 - 30°C is about twice that observed for M. segmentata LDH $Km_{pyruvate}$. $Ea_{oxaloacetate}$ over the range from 5-15°C is 9.85 Kcal $mole^{-1}$ and from 15 - 25°C this rises to 11.93 Kcal $mole^{-1}$. Positive thermal modulation of Km is pronounced and positive thermal modulation of Ea is slight, so oxaloacetate concentration probably regulates MDH activity in the forward direction.

In conclusion, the experiments involving Meta LDH and MDH reported in this chapter confirm that LDH4 band positions on zymograms are heterogeneous, and suggest that the same is true of LDH3. The data do not indicate the relative adaptive values of the allozymes which preponderate in the three species examined in detail by Auld (1976), but they do show that substrate inhibition by lactate in the back reaction and regulation by pyruvate concentration in the forward reaction occur. These observations are consistent with the interpretation that spiders tolerate high levels of lactate during muscular activity. MDH also exhibits positive thermal modulation of Km, but the design of this experiment was inadequate, and little can be concluded about its role in metabolism from the one set of experiments executed.

Chapter 4. The Life Histories of META Species in Scotland.

The results of the October 1973 sample (page 10) clearly demonstrated that M. mengei and M. segmentata are genetically differentiated species. This point is no longer in question, but former interpretations of their morphological and ecological similarities, that they are seasonal varieties or subspecies, implied genetic continuity between sympatric M. segmentata and the smaller mengei form. Because the adult generations of the two forms co-occur in autumn (Chrysanthus 1953), however, these interpretations are untenable: interbreeding to any extent would lead to genetic introgression and dedifferentiation; unless mate recognition systems (Patterson in Press) had evolved, resulting in sexual isolation and, by definition, speciation. The latter is evidently the case.

The genetic demonstration of species status in these siblings led me to investigate their ecologies. All sympatric species have at least one ecological refuge from interspecific competition: the primary objective of the ecological research was to identify such niche differences between M. mengei and M. segmentata and to explain their significance in terms of natural selection and reproductive advantage. Since adult M. mengei and M. segmentata were already well known to be sexually active in different seasons, the obvious starting point was to devise a sampling program aimed at following the life histories of the two species from hatching to egg-laying. The second objective was to investigate the feeding ecologies of the two populations throughout their life histories. This was aimed at showing that breeding asynchrony sets up interspecific size differences, which result in non-overlapping prey-resource utilisation. However, this part of the project was discontinued in August 1974,

partly because it is practically difficult to measure and identify parts of chewed prey items, and partly because the original objective of examining the life histories of two species had by that time been expanded to include M. merianae and M. menardi.

That the study area should support a substantial M. merianae population was not surprising, but my attention was first drawn to a dense population of juvenile spiders which, although they closely resembled the juveniles of the other Meta species, fitted no published description (Locket and Millidge 1953). I briefly entertained the conclusion that these were the juveniles of one of the other species, but this interpretation was inconsistent with the genetics at the LDH, MDH, GOT, and PGI loci. I finally became convinced of their true identities after I found by chance an adult M. menardi which shared the same enzyme phenotype as the juveniles. Later, I began collecting spiders after dark and it became clear that the study area supported also a sizable, though mainly hidden, M. menardi population.

The inclusion of M. merianae and M. menardi in the sampling program has been of immense value in clarifying the "rules" by which spider diversity may be governed. The strongest influences on the evolution of a species arise from competitive interactions with closely related taxa, particularly congeners (Bock 1972), and other factors, such as predator avoidance, which limit the options open to evolving populations. The basis of this principle is that if speciation is to occur, primary sexual isolation and genetic divergence (in allopatry) must be followed by mutual ecological and genetic divergence in sympatry, resulting in secondary sexual isolation (Lewontin 1974). Cases of speciation which have occured in the absence of congeners are known, but such examples raise doubts about the accepted roles of "isolating mechanisms" during speciation

(Patterson in Press), and not about the importance of interactions with functionally similar taxa in the evolution of ecological uniqueness. Thus by identifying patterns of correspondence in their mutual differences, groups of sympatric congeners are valuable material for understanding evolution by natural selection.

This chapter deals mainly with the life histories of the four Meta species, and the microhabitats in which they live. Chapter 5 focuses on the niche differences between them from the reproductive profit / loss point of view, and identifies the nature of each species' refuge zone.

Figure 4.1 The study area: south-east aspect, viewed from a parallel ridge.

Methods.

The study area was situated in a stand of climax oak scrub (Quercus robur) on the shore of Loch Sween, Argyll. This is one of many stands of native woodland which are isolated by conifer forests in this part of West Scotland. As far as is known locally, this particular locality has never been felled, either for charcoal or cultivation, and it is truly climax. The area (National Grid NM 758862-761861) is a ridge approximately 450m long by 45m, pointing south-west into the sealoch and reaching an elevation of 15 - 20m. It is bounded on three sides by water and on the fourth by conifer forest. Figure 4.1 is a photograph of the south-east aspect of the ridge, as it appears from a parallel ridge.

Two principle collecting sites were established. Site 1 was on the broad crest of the ridge where the field layer vegetation is dominated by grasses, bracken, heather, and isolated patches of Luzula sylvatica. The canopy here is thin and uneven and reaches only 4 - 5m above ground level. The ground on the steep north-west slope, Site 2, is largely made up of large rocks, roofed over partly and in places entirely by a thick carpet of Luzula leaves and roots. The canopy is denser and taller, and the field layer more sheltered, shaded and moist here than at Site 1. The south-east slope (Figure 4.1) was occasionally used in preference to Sites 1 and 2 for collecting web data and prey items. The terrain here resembles Site 2, except that its vegetation is typically like Site 1, having no dense Luzula carpet. No significant differences were observed in the age structures of the spider populations sampled at these three sites, and data were pooled, except for species ratio comparisons. Another location, 754872, was also used for after-dark collections of M. merianae and M. menardi. This closely resembles Site 1, except crevices between

large rocks are more accessible here than they are on the main ridge.

Samples were collected from the four Meta populations at 3 - 4 week intervals between April-November 1974, April-November 1975, and March-August 1976. M. mengei, M. segmentata, and some instars of M. merianae and M. menardi, were captured from the field layer vegetation by sweep net. After 20 sweeps the contents of the net were emptied into a plastic tray and every specimen of Meta removed with a pooter. This procedure was repeated until 30 - 100 spiders had been collected, although in some instances sample sizes were smaller, due to unfavourable collecting conditions. Also, most instars of M. merianae and M. menardi were collected from crevices and "microcaves" by searching with torchlight after dusk in 1975 and 1976. Such searches were systematic and not consciously biased. The spiders were kept alive in 6 X 1.5cm clear plastic tubes, then stored in the deep freeze on return to the laboratory.

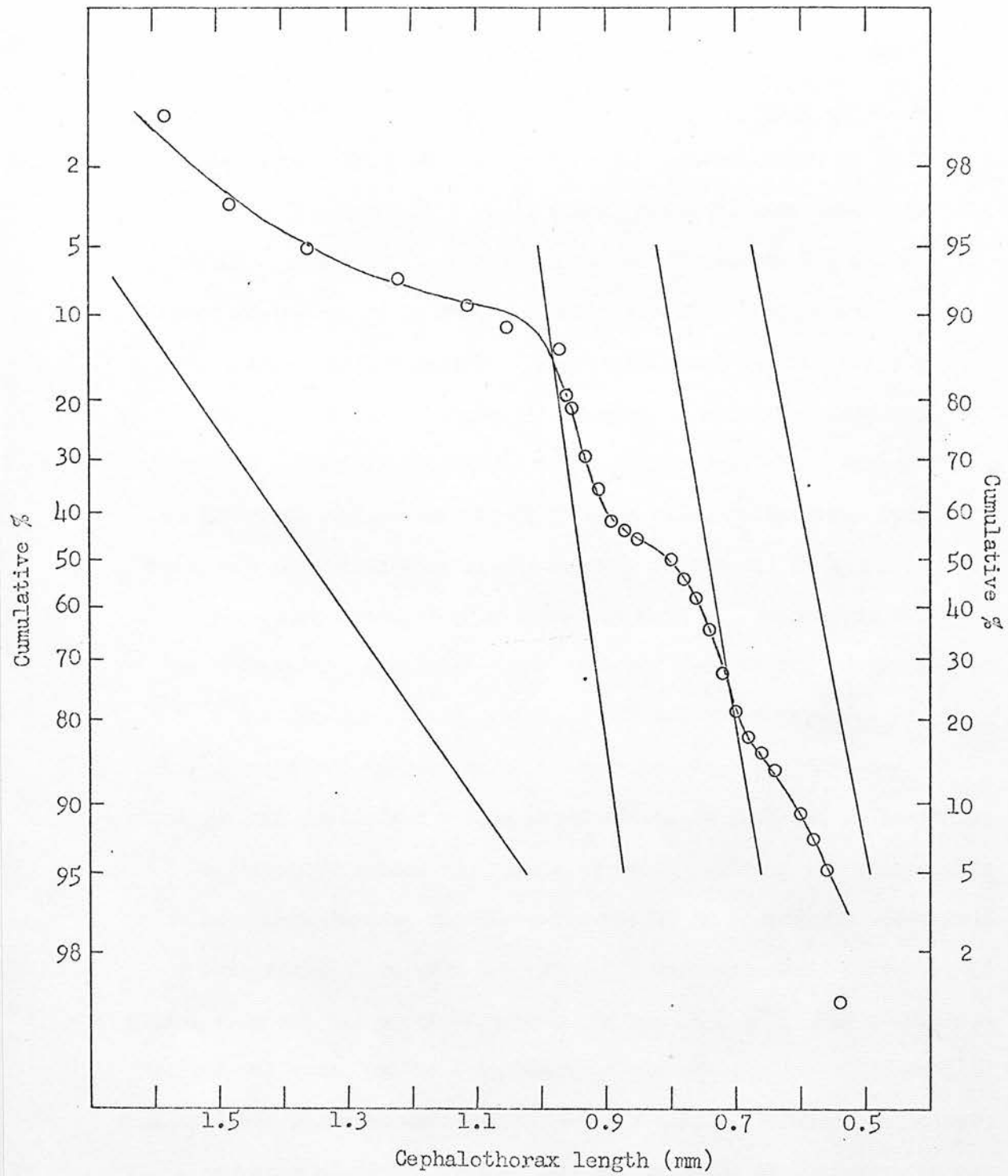
Samples were separated into species and instars by the following procedures. Cephalothorax lengths were measured under a binocular microscope with an eyepiece graticule, and where possible species and sex were noted. Initially, I could identify only adult males of M. mengei and M. segmentata and the larger instars of M. merianae and M. menardi by colouration and morphology. The remaining spiders were prepared for identification by the zymogram method (Chapter 2). Once the data had been grouped by species, the size structure of each population was obtained by graphical probit analysis (Harding 1949) of cephalothorax lengths. This is a simple, robust method for simultanaously obtaining the number of modes in a polymodal frequency distribution, the mean and standard deviation of each mode, and the percentage of the sample attributable to each subpopulation.

In this case, subpopulations correspond to instars; the mean cephalothorax length of each indicates its identity; and the percentage of the sample in each instar gives the size distribution of the population on the sampling date. The method works extremely well even with small sample sizes.

Table 4.1 and Figure 4.2 illustrate a worked example of this method for M. mengei, 7 April 1974. Cephalothorax lengths are first collected in ranks and the sum of observations ($\leq N$) before and including each rank is noted (Table 4.1). Adults need not be included in $\leq N$ because their instar is not in question. In the present sample one juvenile at 1.76mm stands in an instar by itself, and this need not be included either. Cephalothorax length is then plotted on arithmetic probability paper (Chart Well 5571) against cumulative percentage of the sample in and before each rank. The first observation falls at 50 / N% and subsequent observations fall at 100 / N% intervals. There are as many points on the graph as there are ranks. I drew up tables of percentage points for samples up to 100 so that the data could quickly be transferred to probability paper ready for the next step in the analysis.

A smooth curve is temporarily drawn through the points, and points of inflection, where the curve passes a shoulder and begins to rise again, are noted. There is always one more subpopulation than there are points of inflection. In the present sample of 49 juveniles these occur at 14%, 53%, and 90%; so four instars contribute 14%, 39%, 37%, and 10% respectively to the juvenile sample (Figure 4.2). The shape of the first part of the curve (on the right) is determined mainly the smallest instar present, and this must now be expressed in terms of the 14% instar. This is done by dividing arbitrarily chosen points on the curve by 14%. A straight

Figure 4.2 Analysis of a cephalothorax-length frequency distribution by the method of Harding (1949). M.mengei (juveniles) 7 April 1974.



Solution:	10%	37%	39%	14%
	1.36±0.2	0.94±0.05	0.74±0.04	0.58±0.05

Table 4.1 Analysis of a cephalothorax-length frequency distribution by the method of Harding (1949).
M.mengei 7 April 1974.

Rank	CL(mm)	N	ΣN	%	Rank	CL(mm)	N
1	0.54	1	1	1.0	28	1.76	1
2	0.56	2	3	5.1	29	1.84	1(♀)
3	0.58	1	4	7.1	30	1.92	1(♂)
4	0.6	1	5	9.2	31	1.94	1(♀)
5	0.64	2	7	13.3	32	2.0	4(1♂,3♀♀)
6	0.66	1	8	15.3	33	2.08	7(3♂♂,3♀♀)
7	0.68	1	9	17.4	34	2.16	6(4♂♂,2♀♀)
8	0.7	2	11	21.4	35	2.24	5(1♂,4♀♀)
9	0.72	3	14	27.6	36	2.32	1(♀)
10	0.74	4	18	35.7			N=76
11	0.76	3	21	41.8			
12	0.78	2	23	45.9			
13	0.8	2	25	50.0			
14	0.85	2	27	54.1			
15	0.87	1	28	56.1			
16	0.89	1	29	58.2			
17	0.91	3	32	64.3			
18	0.93	3	35	70.4			
19	0.95	4	39	78.6			
20	0.96	1	40	80.6			
21	0.97	3	43	86.7			
22	1.05	1	44	88.8			
23	1.11	1	45	90.8			
24	1.22	1	46	92.9			
25	1,36	1	47	94.9			
26	1.48	1	48	96.9			
27	1.54	1	49	99.0			

% at rank 1 = 50/N, interval = 100/N

Solution, including ranks 28 - 36:

9%	25.2%	23.8%	6.5%	1.3%	34.2%
0.58±0.05	0.74±0.04	0.94±0.05	1.36±0.2	1.76(1)	2.1±0.12

line is usually found with 3 - 5 points obtained in this way; this is the normal distribution of cephalothorax lengths of this instar.

Up to this point, the curve drawn through the data has been an estimate of the resultant's true position. The first part of the resultant can now be drawn in by reversing the procedure above, and multiplying the first straight line by 14%. The position of the line should be revised until a good fit with the data is obtained. However, if this procedure was continued indefinitely, a plateau would be reached at 14%. The difference between the resultant drawn from the first instar present and the estimated curve is due to the second instar present. Thus by subtracting the contribution of the first instar from the total (and this soon reaches its maximun of 14%), points on the second straight line can be found by dividing by 39%. The procedure is repeated for each instar and the positions of the lines, and percentages if necessary, are revised until the best fit with the data is obtained.

Finally, the means of the distributions are read of the graph where they cross 50%, and the standard deviations are found from the interval between where the line crosses 50% and 15.87%, this being the definition of the standard deviation of the normal distribution. The adult mean cephalothorax length is calculated arithmetically, and the percentages in the juvenile instars are revised to take the size of the whole sample into account. Data obtained by this method appear in Appendix 3.

Most of the spiders collected in 1976 and some which were left over from previous years were used to estimate dry weights of the instars. By this time I was able to recognise subtle colour differences between most M. mengei and M. segmentata. Cephalothorax lengths were measured and each spider was wrapped separately in a

3 X 3cm square of aluminium foil which had been preweighed and numbered. Samples were dried at 70°C for four days, then reweighed on a Cahn Gram Electrobalance. Before and after treatment foils were weighed an the 10mg scale with tares of 40, 50, 60mg, or more, depending on the absolute weight of foil + spider. Weights were recorded to an accuracy of 10^{-2}mg.

Mean dry weights were estimated by first dividing each species-sample into instars by the graphical method. Most instars contained more than 20 measurements . 95% confidence limits were marked around the instar means on the basis of $t(\alpha=0.05) = 2.0$ (standard deviations). Very few individuals had to be assigned to instars arbitrarily. Cube root dry weight was first regressed on cephalothorax length within each instar, but few regression lines differed from 0, and mean instar cephalothorax lengths and mean dry weights$^{\frac{1}{3}}$ are used instead.

Feeding ecology was studied between April and August 1974. This data has not been analysed quantitatively, but conclusions are drawn from it in Chapter 5. The problem in the field was obtaining a random sample of prey from each instar on each sampling date. Adults reduce small prey items to unrecognisable fragments in a matter of minutes; adult webs were located and marked with garden canes, and visited at 10 - 15 minute intervals to overcome the large-prey bias which would be introduced by a random search routine. Prey were removed from the adults' jaws, stored in tubes and labelled. The adults were also collected, and measurements made of their webs at the end of the sampling period. This procedure is very unproductive for juveniles, and random searches were adopted to collect these data. Adjoining plots of vegetation were searched for webs, and the number of webs sighted before finding a feeding

Meta noted. The number of web radii, its diameter (mean of vertical and horizontal axes), and its angle to the horizontal were recorded for most webs; inter-spiral distances were measured on others. The feeding spiders and their prey were stored in separate collecting tubes and labelled. These procedures were designed to estimate the relative feeding frequencies of each instar, and the mean prey size and variance taken by each instar. Insects were collected from the routine sweep-net samples. These were to be used for comparing the prey taken with the background prey size distribution. The size structures of the spider populations and the species ratios obtained by sweep-sampling would have given expected frequencies for comparison with observed feeding frequencies.

Results and Discussion.

Table 4.2 reports weighted mean cephalothorax lengths and standard deviations of all the instars of the four species identified by the graphical method. Meta species moult once in the eggsac and the instars are numbered accordingly. (Sample: one each of M. mengei and M. segmentata eggsacs; after hatching and leaving the eggsacs, the numbers of 2nd instars equalled the numbers of exuvia). There is no significant cephalothorax sex dimorphism in these species and the sexes are pooled. Figure 4.3 illustrates the change in linear size at each moult. The average growth factors for linear size (nth / (n-1)th instar) are 1.31, 1.29, 1.37, and 1.35 respectively for M. mengei, M. segmentata, M. merianae, and M. menardi. These figures are somewhat higher than those for insects (Bodenheimer 1933), but there is certainly no latent moult. Therefore the absence of 5th instar M. menardi from the samples most probably represents sampling error, rather than a peculiarity in the growth pattern of this species. Table 4.3 reports mean dry weights$^{\frac{1}{3}}$ of some Meta instars, and Figure 4.4 shows that mean dry weight$^{\frac{1}{3}}$ is a linear function of mean cephalothorax length (adult males were omitted from the regression line because, as Figure 1.2 shows, they are shaped differently from females and juveniles). Therefore, cephalothorax length is a good measure of linear size.

In Figures 4.5 - 4.7 percentage of sample in the nth instar is plotted against time. Each point is the weighted mean percent over three years at coincident or near-coincident dates. Points within each instar are joined in smooth curves, showing rhythmic rise and fall of instar frequency. The sum of frequencies at any time is 100%, so sampling error has been corrected for in noisy instars by obtaining estimates for such curves from the smoother, more

Table 4.2 Mean Cephalothorax lengths and standard deviations (mm).

Instar	M.mengei	M.segmentata	M.merianae	M.menardi
2	0.55±0.04 (133)	0.62±0.053 (65)	0.61±0.042 (84)	0.77±0.05 (169)
3	0.715±0.055 (263)	0.85±0.065 (42)	0.79±0.052 (96)	1.04±0.06 (62)
4	0.935±0.078 (224)	1.17±0.059 (26)	1.15±0.146 (104)	1.39±0.14 (12)
5	1.25±0.09 (111)	1.37±0.036 (17)	1.49±0.188 (70)	--
6	1.66±0.132 (113)	1.67±0.098 (22)	2.02±0.76 (29)	3.97±0.75 (2)
7	2.09±0.215 (119)	2.15±0.29 (43)	2.9±0.934 (33)	5.45±0.17 (16)

Figure 4.3 Mean cephalothorax lengths of instars.

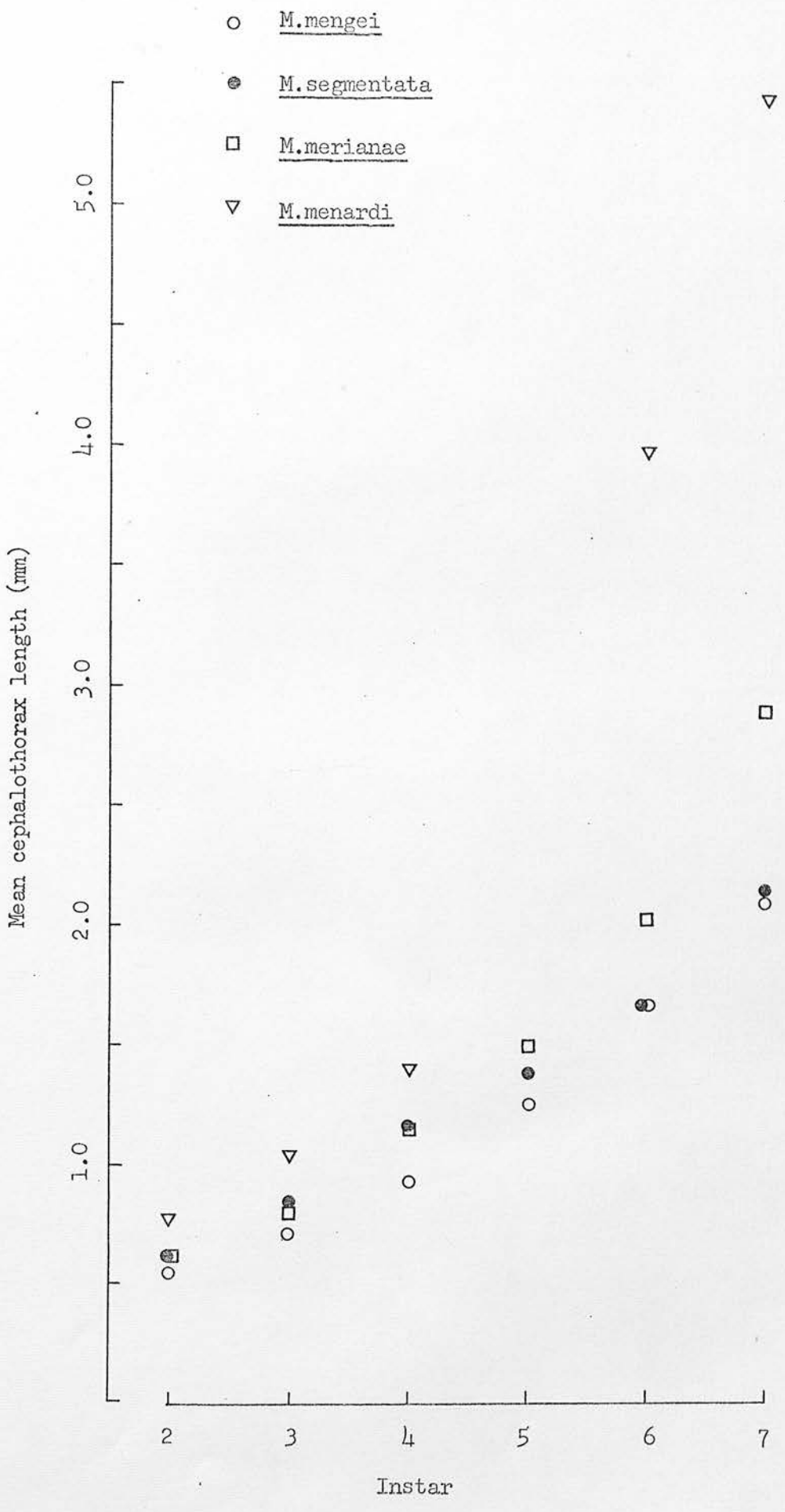
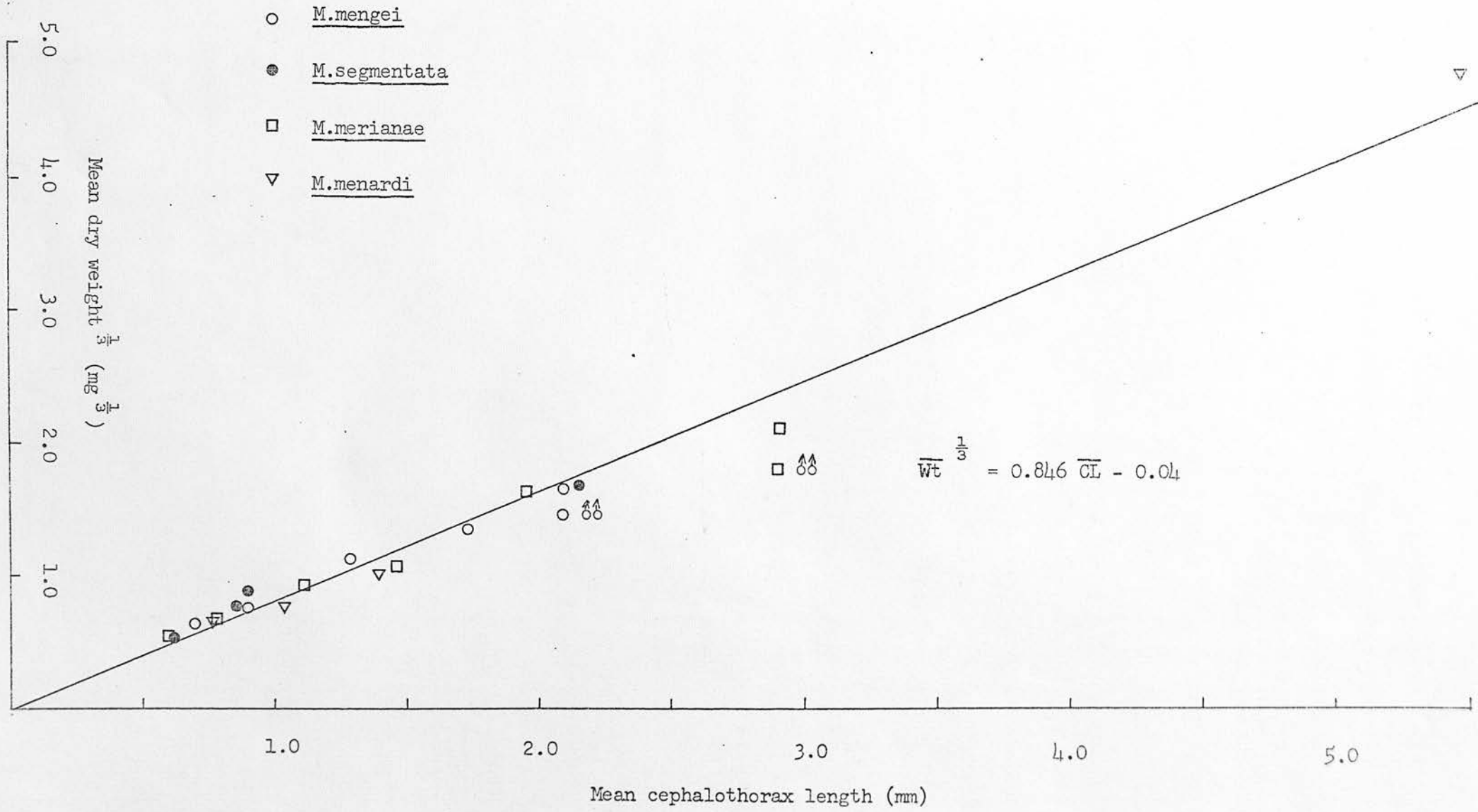


Table 4.3 Mean dry weights $^{\frac{1}{3}}$ and standard deviations ($mg^{\frac{1}{3}}$).

Instar	M.mengei	M.segmentata	M.merianae	M.menardi
2	--	0.54±0.24 (4)	0.56±0.09 (43)	0.69±0.18 (15)
3	0.65±0.11 (30)	0.78±0.16 (5)	0.68±0.11 (48)	0.78±0.13 (9)
4	0.77±0.09 (52)	0.9±0.09 (4)	0.94±0.11 (32)	1.01±0.11 (9)
5	1.13±0.16 (81)	--	1.09±0.18 (35)	--
6	1.37±0.18 (20)	--	1.64±0.35 (41)	2.06 (1)
a♀♀	1.67±0.28 (24)	1.68±0.44 (23)	2.11±1.51 (15)	4.79±0.64 (6)
a♂♂	1.48±0.08 (4)	--	1.82±0.2 (9)	1.28 (1)

Figure 4.4 Regression of mean dry weight $^{\frac{1}{3}}$ on mean cephalothorax length.



reliable instars. Considering the biological nature of the data - that instar frequency is expected to change progressively rather than erratically - this is quite permissible. Data for M. menardi are incomplete and cannot be graphed meaningfully. These appear in Table 4.6, which is abbreviated from Appendix 3. No significant differences were detected in the phenologies of the sexes and sex ratios appear only in the Appendix.

Spider sampling by sweep net does not provide reliable measures of density (Turnbull 1960), especially where web sites are patchy and foraging behaviour is influenced to some extent by weather conditions and the time of day. I omitted estimates of density from the sampling program because to have tackled this problem seriously would have involved expressing densities in terms of numbers per unit of vegetation volume, times some weighting factor which took into account the density of potential web sites on each type of floral substrate. Geyer (1971) obtained good density estimates for M. segmentata by cutting volumes of vegetation into a bag on the ground, thereby sampling more than just the surface area of the field layer. However, the field layer vegetation at the two principle collecting sites in the present study was much too short to uses Geyer's "bagging" method: too much disturbance would have been created while placing the bag in position beneath the vegetation. A few measures of density were made by marking out areas with garden canes and sweeping within the boundaries: these showed that at Site 2, Meta densities varied between 1.5 spiders m^{-2} in April 1976 to 2 spiders m^{-2} in June. Niether of these truly describes the microdistribution of orb webs. Table 4.4 reports species ratios, and I shall qualify these with subjective statements about densities (eg. scarce, abundant, etc.).

Table 4.4 Species composition of field layer sweep samples.

	Site 1			Site 2				
Mean date	M. mengei	M. segmentata	N	M. mengei	M. segmentata	M. merianae	M. menardi	N
3 March	100%	--	27					
7 April	100%	--	96					
27 April	100%	--	103	50%	--	50%	--	46
20 May	93%	7%	69	2%	--	10%	89%	114
11 June	35%	65%	39	18%	12%	26%	44%	34
2 July	60%	40%	85	28%	19%	31%	22%	87
21 July	65%	35%	40	18%	8%	50%	24%	54
10 Aug	75%	25%	118	42%	16%	34%	8%	58
5 Sept	50%	50%	24					
27 Sept	81%	19%	52	31%	--	69%	--	64
22 Oct	96%	4%	54	29%	32%	39%	--	28
3 Nov	96%	4%	76	27%	9%	64%	--	11
19 Nov	90%	10%	19	53%	33%	14%	--	15

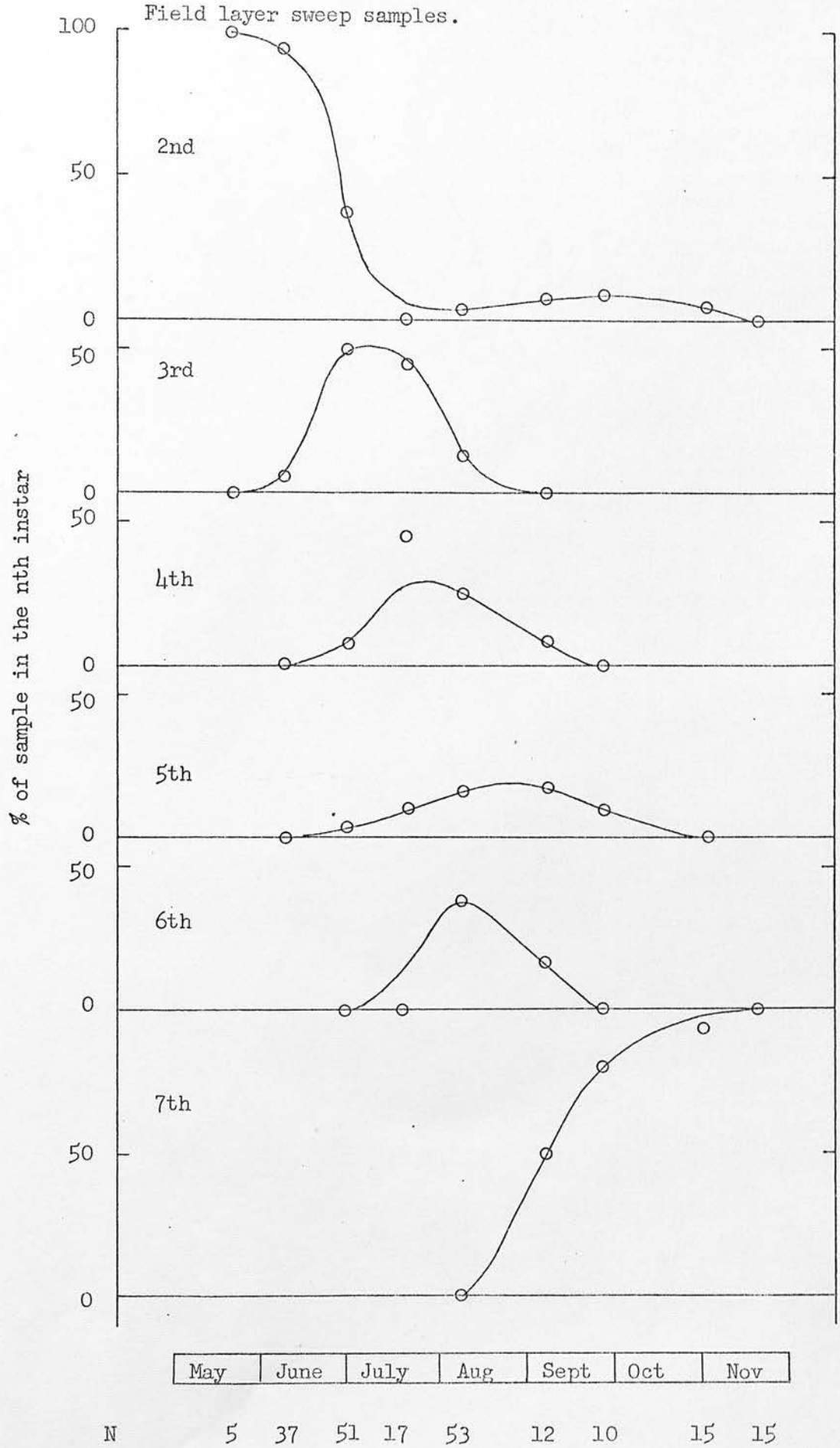
Table 4.4 and Figure 4.5 show that the first M. segmentata hatchlings appear in the field layer towards the end of May. By the end of June the hatch is virtually complete, and already some of the population has passed into the 5th instar. Growth continues rapidly into the 6th instar, and in late August the first adults appear. From mid-September onwards the frequency of adults in the population is prevented from reaching 100% only by a few individuals which have either hatched late or have somehow been trapped by starvation in the 2nd instar.

Young M. segmentata spin their webs mainly on heather, under bracken fronds, and among the leaves of Luzula, but adults especially favour tall grasses, the dry flower heads of Luzula, and other tall substrates. Dabrowska-Prot and Luczak (1968) comment that M. segmentata move to the upper stratum of the field layer as they reach maturity in August, but no other author appears to specifically mention this.

My impression is that most successful courtships happen from late-September to mid-October, and that their attendance at the webs of females into November reflects the males' perserverence in their search for virgin females (Bristowe 1958). The adults die over winter. Eggsacs are built amongst roots and under stones, as are the eggsacs of M. mengei and M. merianae.

Adult M. mengei co-occur with M. segmentata adults in early autumn. Chrysanthus (1953) recognised that co-occurence as sexually mature adults distinguishes true species from varieties and he elevated M. mengei to species status accordingly. However, my data from October 1973 indicate that M. mengei does not normally engage in reproduction in autumn. 69 adult males and 111 adult females were individually collected. 54 were found in pairs and

Figure 4.5 Seasonal changes in size structure: M. segmentata.



all 27 pairs were M. segmentata. None of the 9 adult male or 21 adult female M. mengei was involved in courtship. Chrysanthus (1953) mentions that adult male M. mengei have been found courting M. segmentata females in autumn, but this is probably unusual.

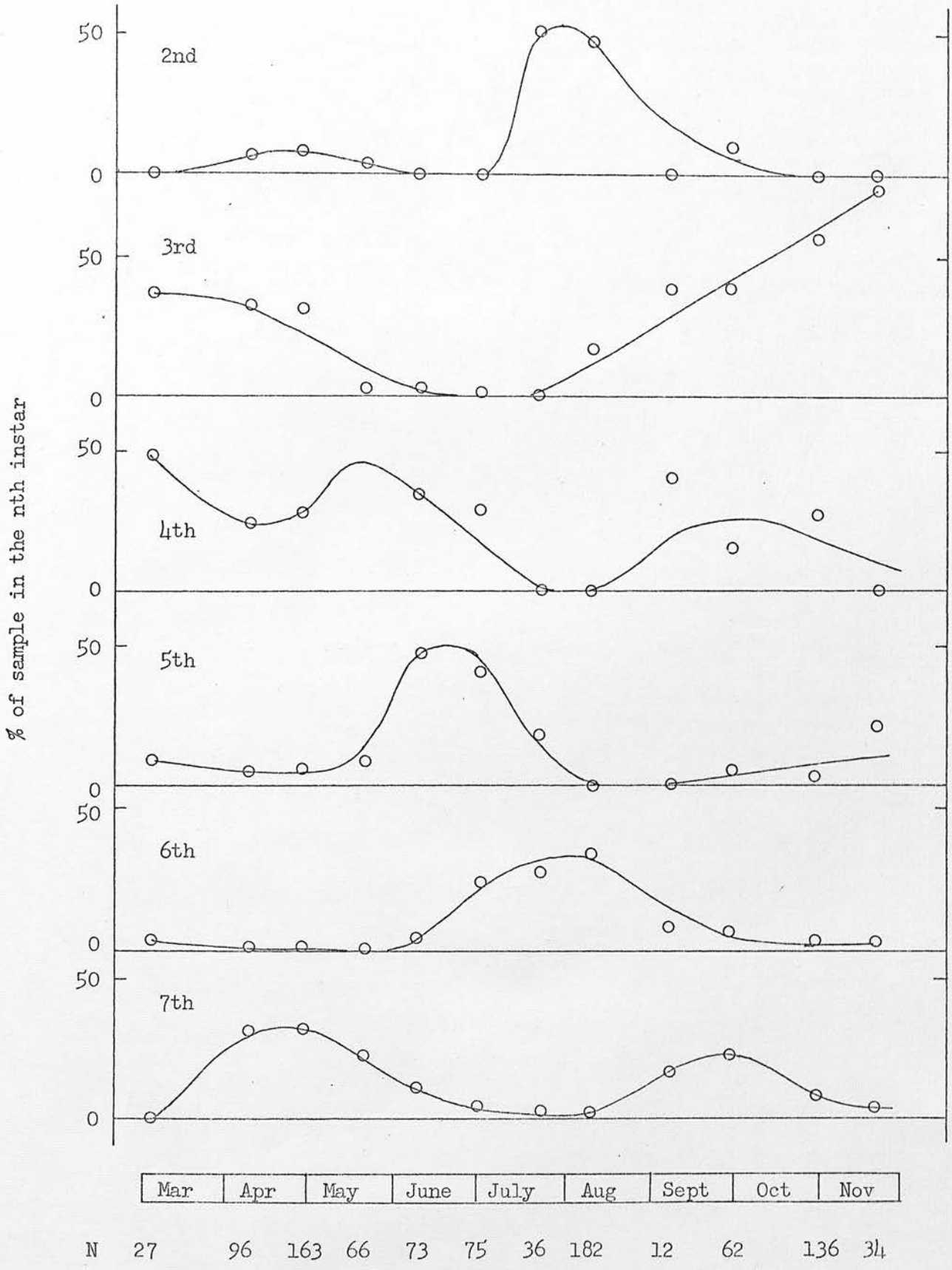
M. mengei mates in April in Argyll, after having overwintered mainly in the 7th instar. The timing of the breeding season varies widely from April to June in Scotland, depending on the location. Argyll is mild and breeding begins sooner there than it does in Eastern Scotland, although age structures are very similar on both sides of the country. The new generation hatches during the following late-July to early-August, and by late-September the hatchlings have virtually all moulted once (Figure 4.6). Most of this generation overwinters for the first time in the 3rd instar and growth into the 4th instar recommences in the following March-April. Peak frequencies of 4th, 5th, and 6th instars occur between May and August, and this generation reaches adulthood 13 - 14 months after hatching, overwinters for the second time, and mates in April, two years after the parental generation.

The small number of second instar M. mengei recorded in April and May probably represent spiders which either hatched too late to moult before winter or which caught insufficient food to moult in autumn, rather than the offspring of autumn-breeding adults. They correspond with the 2nd instar M. segmentata which never grow beyond that stage.

The dip in adult M. mengei frequency observed between early-October and late-March shows that adult M. mengei move out of the volume sampled by a sweep net, whereas the younger generation continues to forage actively later in the year, and begins foraging sooner than the adult generation: the two generations become

Figure 4. 6 Seasonal changes in size structure: M. mengei.

Field layer sweep samples.



vertically stratified. Edgar (1971a, 1971b) demonstrated that juvenile Pardosa lugubris overwinter below subadults in the litter layer. He attributed this to an adaptation for countering cannabalism of the smaller by the larger instars. However, this is an "evolution for the good of the species" arguement which can hardly be taken seriously, unless it could also be shown that all of the spiders in an area were closely related by parentage. Such a situation could arise if adult females always returned to the same places to lay both of their clutches; and if family groups are geographically viscid (Seger 1976) over several generations. This is certainly not the case in biennial M. mengei populations, because the degree of relatedness between co-occuring generations is proportional to the frequency of annuals in the population; and even then the genetic exchange is one-way, from the younger to the older generation. Under the observed conditions, it would not be of advantage to a spider's genes not to eat its neighbour. The simplest explanation of the vertical stratification is that web spinning is costly even though feeding may be profitable; and for an adult female the rewards must be high before it is profitable to begin the process of reproduction, whereas for a small spider more is to be gained by foraging under unfavourable conditions.

As far as I could tell, juvenile M. mengei and M. segmentata occupy similar web sites, although Table 4.4 shows that the ratio of the two species in June differs between Sites 1 and 2. This suggests that juvenile M. segmentata may be recruited from the more open and exposed Site 1 to Site 2, or that hatching at the cooler Site 2 is slightly behind Site 1. If anything, the species ratios show that adult M. segmentata are commoner (relative to M. mengei) at Site 2 than they are at Site 1 in autumn. Palmgren (1972, 1974) identified horizontal separation between these species, M. mengei

Table 4.5 *M. mengei* / *M. segmentata* ratios at two collecting sites in Eastern Scotland (11 September 1975).

	Periphery		Within-wood	
Instar	*M. mengei*	*M. segmentata*	*M. mengei*	*M. segmentata*
2	4%	--	13.5%	--
3	23%	--	25%	--
4	17%	--	13.5%	--
5	23%	--	13.5%	5.5%
6	8%	7%	--	5.5%
7	23%	93%	37.5%	89%
N	26	15	8	18
ratio	1.73		0.445	

being shifted over towards damper and more shaded areas (represented here by Site 2) relative to M. segmentata in Finland. In Denmark, the M. segmentata / M. mengei species ratio changes almost from 1 to 0 along the axis from clearing to open beechwood to dense beechwood to dense spruce (Toft pers. com.). Conversely, British collectors (Geyer 1971; A. Lindley pers. com.) find that M. segmentata is usually more common in shaded parts of woodland than it is at the periphery of woods. I specifically tested for horizontal separation with two sweep samples taken from mixed deciduous woodland in Eastern Scotland. The results are reported in Table 4.5. They show that the age structures of the populations at the two sites were similar, but that M. mengei out-numbered M. segmentata at the periphery of the wood and that the reverse was the case inside the wood. Taken at face value, these data and the trend in the autumn species ratios at Sites 1 and 2 in Argyll contradict Palmgren (1972, 1974) and Toft. However, it is certainly the case in Scotland that the unequal species ratios reflect trends in vegetation height rather than an exposed / sheltered web site preferences of the spiders. Adult female M. segmentata show distinct preferences for tall, isolated stems such as Luzula or willow herb to spin their webs on, and they are consequently to be found in greater numbers wherever such web sites are more frequent in the field layer, irrespective of whether these are at the periphery or within woodland. There may also be a exposed / sheltered trend, but this is swamped by vegetation height trends, in Scotland at least.

M. mengei and M. segmentata are diurnal foragers which are commoner in more exposed parts of the field layer, but which also occur on the shaded slopes of the ridge, represented by Site 2.

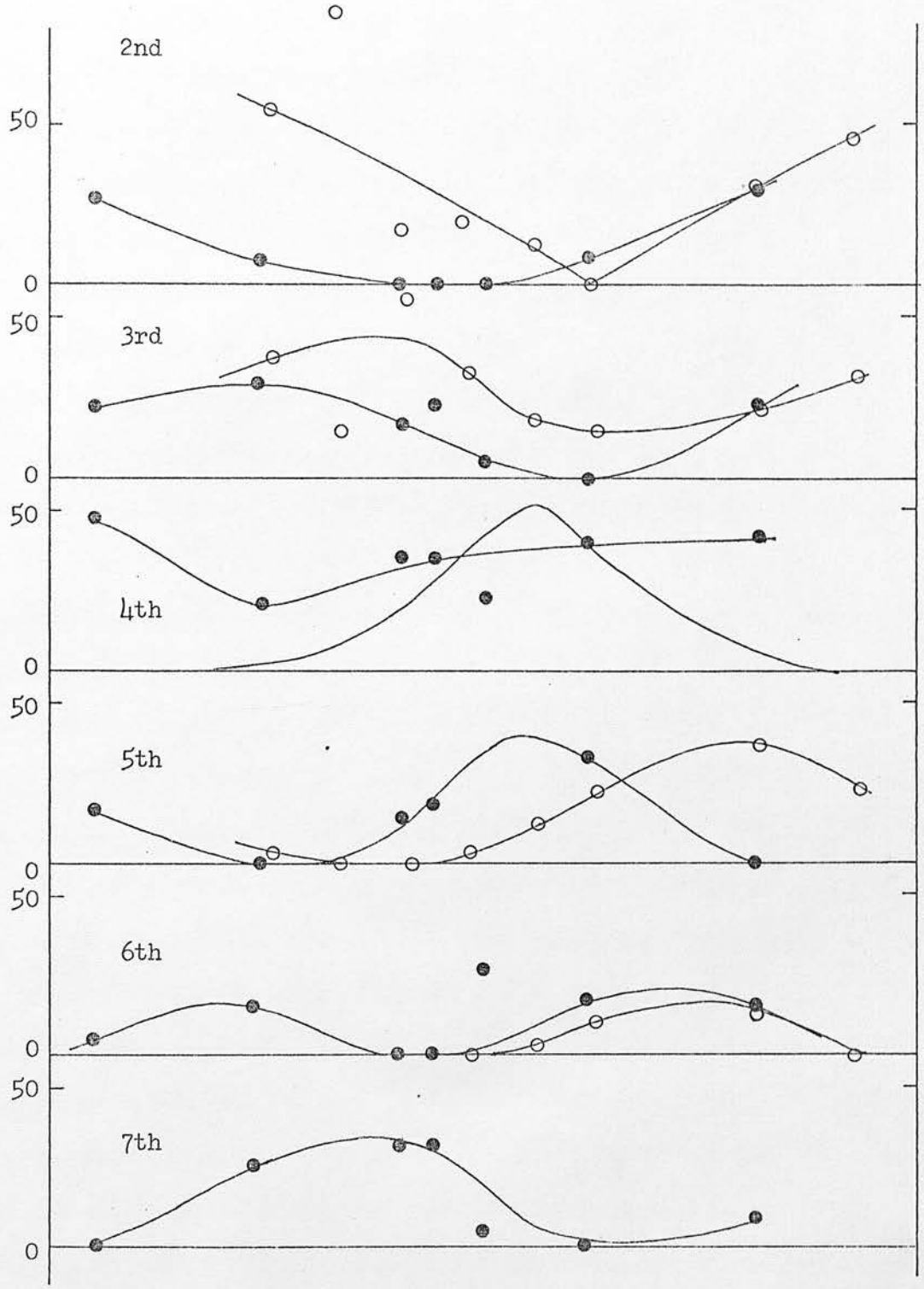
M. merianae and M. menardi, on the other hand, are nocturnal at some stages of their life cycles, and these species are confined to the shaded and uneven slopes of the ridge (Table 4.4). Here I recognised three Meta microhabitats. M. menardi instars 2 and 3 and juvenile M. merianae forage by day in the field layer, alongside M. mengei and M. segmentata. Adult M. merianae occupy web sites under overhangs of rock and vegetation, in shallow gaps and crevices between rocks, and at the entrances to deep hollows between rocks and the ground. They do not occur in sweep samples because they are active only at night, but they can be shaken from overhanging vegetation. Adult M. menardi are confined to the deepest crevices and hollows, situations which are best described by the word "microcaves". The adults of this species are strictly nocturnal, and unlike adult M. merianae they do not require overhanging vegetation to hide in during the day.

Figure 4.7 summarises the M. merianae data obtained by the two sampling methods. This Figure shows that there is a phase-lag of 1 - 2 months between events in the after-dark crevice microhabitat and the field layer populations. This does not reflect differences in growth rates between the two locations, or an artefact of the sampling procedures, but it illustrates that newly-hatched and newly-moulted juvenile M. merianae linger for a time in the crevice microhabitat, where they return to moult, before migrating into the field layer to forage by day in the vicinity of the adults' microhabitat. Young M. merianae collected at night were rarely on whole webs, and usually showed no inclination to web-build. Nor could juveniles be found in the crevice situations during the day in the numbers with which they occured at night. Hence there is close agreement between each field layer curve in Figure 4.7 and the succeeding after-dark

Figure 4.7 Seasonal changes in size structure: M. merianae.

○ Field layer sweep samples.

● After-dark collections.



○N			54	11	28	27	28	19		44	18
●N	36		24		28	41	38	11		31	

curve, because these represent spiders of similar ages but different sizes. The after-dark curve probably samples spiders which are on their way to the field layer after a period of seclusion and moulting.

This species hatches in late-July through September and overwinters for the first time in the 2nd and 3rd instars. By the following July most of this generation is foraging in the 4th instar and those which moulted into the 5th instar over June-July arrive in the field layer in early autumn. The second winter is passed mainly in the 6th instar, though some adults appear in autumn. The dip in 6th instar frequency between October and March and the rise of adult frequency from zero% in the spring indicates that the larger instars of this species, like adult M. mengei, move out of the volume sampled by my methods and become inactive earlier in the autumn than smaller instars do. Again, the reason for this behaviour may be that it is not worthwhile,in terms of the costs of foraging versus the benefits, for large Meta to spin webs over this period. The final moult takes place mainly in spring and the adults mate in May and June. By July most of this generation has died and, as in M. mengei, there is probably no opportunity for genetic exchange between successive generations, although they temporarily mix in the 3rd instar field layer population and 4th instar after-dark population in summer (neither curve reaches zero%).

Turnbull (1960) classed M. merianae as a field layer species, presumably because only juveniles were represented in his samples, and Cherret (1964) described the microhabitat of adult M. merianae as being confined to overhangs of peat in drainage channels of his moorland study area. He found that juveniles spun their webs amongst Calluna and that they were not confined solely to the overhang situations. He also demonstrated that juvenile M. merianae are

positively phototactic and that adults are negatively phototactic, and suggested that this behavioural difference between the instars accounts for selection of different microhabitats by juvenile and adult M. merianae. However, it is not simply that adults select dark or shaded places in which to spin their webs, but also that they are nocturnal as opposed to diurnal. It is true that their webs can normally be found, as I put it on page 1, at the transition from open field layer to situations of near-darkness, but it is also the case that adult M. merianae do not normally respond to stimulation of their webs by a tuning fork during the day, even though they remain in touch with the hubs of their webs via signal threads. At dusk they become active, the females assuming the head-down foraging posture at the hubs of their webs and the males searching for and courting females. New webs are usually spun within two or three hours of darkness.

Palmgren (1972, 1974) also records juvenile M. merianae from field layer vegetation and adults from overhangs of vegetation and rock, but in Finland adult M. merianae appear only in autumn and it is not clear from Palmgren's (1974) data exactly how the life histories of Scottish and Finnish M. merianae populations differ.

M. menardi is usually recorded as living only in caves, but in Argyll the young disperse from the deep hollows where they hatch in late-April and early-May and forage by day in the field layer (Tables 4.4 and 4.6). The hatch is as abrupt as are the M. mengei and M. segmentata hatches, and is about three weeks in advance of M. segmentata. By late-May 2nd instar M. menardi numerically dominate the field layer at Site 2. They disperse more widely than juvenile M. merianae, as far as the ridge crest, where the vegetation changes from Luzula carpet to grasses and braken.

Table 4.6 Seasonal changes in size structure: M. menardi.

Collection date.	% of sample in nth instar						
	2nd	3rd	4th	5th	6th	7th	N
	field layer sweep samples						
25 April	100%						2
20 May	100%						101
9 June	82%	18%					63
27 June	42%	47%	5%				29
10 Aug			100%				5
	after-dark collections (numbers)						
25 April						1	1
20 May						3	3
6 June		5	1			6	12
17 June		28				5	33
4 July			9	1		1	11
4 Aug			1		2		3
26 Sept		5	1			1	7
14 Oct					1		1

Growth through instars 2 and 3 is rapid and by mid-August this species is no longer found in field layer sweep samples.

I did not especially concentrate on collecting juvenile M. menardi in after-dark samples early in the year because it seemed that for these spiders, crevices represent the periphery of the field layer: there was no indication that they return there for a period of seclusion at the moult from 2nd to 3rd instars. They grow quickly and are apparently restricted much less than juvenile M. merianae are to the field layer in the vicinity of adult M. merianae web sites, and I believe that the first free-living moult of this species takes place in the field layer. At this stage, 2nd and 3rd instar M. menardi have distinct black and white dorsal patterns (Figure 1.1), but at the moult from 3rd to 4th instars, which probably takes place in the crevice microhabitat, the white regions of the pattern darken to dark brown and the spiders become nocturnal, seek out the microcaves in which they mature, and are virtually lost to my sampling methods. The colour change and switch of foraging cycle also occur in laboratory populations of M. menardi.

From the scant data in Table 4.6 the following interpretation of the remainder of M. menardi's life cycle seems reasonable. The average growth rate continues unabated in the nocturnal juvenile population, and 3rd instar spiders which left the field layer in late-June are in the 6th instar by September. One adult was found in late-September, but the final moult takes place mainly in spring. The adults mate in May and June and their offspring hatch from their eggsacs in the following April. Thus although this species may mature within one year of hatching, the life cycle is two years in length. As in M. mengei and M. segmentata, some juvenile M. menardi are slow developers: these were detected in the late-September

after-dark samples.

Fourth instar and adult M. menardi were observed with equal frequencies in after-dark collections, but the two instars in between these are poorly represented in the data. The only 5th instar I observed appears in the centre photograph of Figure 1.1; but even that is an estimate of its size, because the spider dropped from its web and did not return after the first electronic flash. It is my impression, therefore, that the 5th and 6th instars are spent in situations well away from daylight, perhaps several meters from the field layer, in deep microcaves. Most 4th instars may have been collected while they were migrating to such situations from the field layer - microcave transition zone (ie. the crevice microhabitat) where they moulted, and the comparatively high frequency of adults in the samples may largely be due to greater mobility, and hence wider dispersion within microcaves, during the breeding season.

From my point of view, M. menardi-containing hollows were few and far between, and typically inaccessible. It is probably true that I was aware of only a small proportion of these situations in the parts of the study area over which I searched. One such location which I returned to several times was a hollow between large rocks and the ground. The rocks were set into a wooded slope and were covered by Luzula carpet. The main entrance was about 1m wide by 50cm high, and the chamber tapered to about 30cm high at the back wall, 2m from the entrance. Here several M. menardi eggsacs were suspended from the roof. 50cm into the hollow, at one side, a 20 X 30cm opening led into a second, larger compartment which curved out of sight. On more than one occasion, adult females and courting males were on webs in front of this second opening, and within the larger chamber were other adults and eggsacs.

The lower photograph of Figure 1.1 was taken with a 210mm lens from the entrance to this second chamber. The photograph of the 5th instar M. menardi was taken at a location nearby. This chamber was very much smaller, about 1m deep with a 20 X 20cm entrance, but the photograph shows that there is also a small opening to another chamber, behind the spider. I never observed the larger instars or adults of M. menardi in these situations during the day, and I presume that they retreated into deeper chambers, well away from daylight. There is probably a much larger underground M. menardi population which the observer on the surface can never sample.

These, then, are the principle events in the life histories of the four Scottish Meta populations. They differ from the life histories implied by Locket and Millidge (1953) in two main respects: M. menardi juveniles are diurnally active and are not confined to caves; and M. mengei, M. merianae, and M. menardi have two-year life cycles in Scotland. However, spider species commonly adopt biennial life cycles at high latitudes and high altitudes (Almquist 1969; Toft 1976). The principle ecological differences between the four adult populations are summarised in Figure 4.8. M. mengei, M. merianae, and M. menardi all breed in spring or early summer, but M. segmentata breeds in autumn and is separated from the other species along the breeding season niche dimension. The sibling species are separated from one another in autumn in vertical space as a result of M. segmentata's preference for tall web sites, and they may also be separated by a horizontal space component (Palmgren 1972, 1974; Toft pers. com.). However, there is overlap along both of these dimensions. The field layer siblings are separated from

Figure 4.8 Niche differences in pairwise comparisons of adult Meta species.

	M. mengei	M. merianae	M. menardi
M. segmentata	Season Horiz., Vert. space	Season Horiz.space Diel cycle	Season Horiz. space Diel cycle
M. mengei		Horiz. space Diel cycle	Horiz. space Diel cycle
M. merianae			Horiz. space

adult M. merianae in horizontal space, and M. merianae is in turn separated from adult M. menardi along this dimension. The latter two species are active only during the hours of darkness, and the sibling species are separated from them along the diel time dimension.

Chapter 5. Competition, Predation, and Resource Partitioning.

Sympatric species are more frequently separated by spatial (habitat) and foraging manner (food type) niche differences than by temporal niche differences, although the relative importance of each of these categories varies among taxa. For instance, temporal differences are more frequent between visual predators whose prey themselves may become time-specialists in order to avoid predation; in animals with relatively limited abilities to use or process available food; and in ectotherms whose activities are sensitive to climatic variations (Schoener 1974a, 1974b). Thus, whereas sympatric bird species partition resources along the horizontal and vertical space and food type dimensions (Cody 1968), sympatric lizards exhibit overdispersion along the diel time dimension also (Pianka 1969); even within species, where a temporal distribution of territories may improve the feeding efficiency of each territory holder (Simon and Middendorf 1976). Figure 4.8 showed that between them, the four adult Meta species occupy three zones in horizontal space, two zones on the diel time continuum, and two breeding season zones. This chapter is concerned with identifying which of these niche differences form the boundaries around the species' refuges from interspecific competition, and which are primarily the results of predator avoidance.

The spatial niche differences between the species primarily follow the boundaries between non-overlapping prey populations. M. mengei and M. segmentata overlap spatially and they share an essentially common pool of diurnal insects, mainly Diptera. They are spatially and temporally separated from adult M. merianae and M. menardi and do not directly compete with these spiders for prey. In Argyll, the latter species are sexually active when their

respective microhabitats are potentially at their most productive. In late-May and June several species of Tipulidae shelter in crevices and microcaves and under overhangs when the temperature falls at dusk, and both nocturnal spider species utilise these prey. However, the locations and angles of their orb webs ensure that they also utilise non-overlapping prey populations. The webs of adult M. merianae are normally spun close to the vertical plane (Figure 5.1d), and for this reason they sample prey mainly on to-and-from crecice, microcave, and overhang flight paths. M. menardi adults' webs, on the other hand, are usually spun close to the horizontal plane, and these are better placed to catch food items which fall from the ceilings and walls of microcaves, such as isopods and other crawling arthropods, than prey types which fly on horizontal flight paths.

Relative to their young, which spatially and temporally overlap with M. mengei and M. segmentata in the field layer, M. merianae and M. menardi adults are specialised along the habitat and diel time dimensions. According to contingency models, which weigh the gains from utilising a unit (of space, food, or time) against the gains from skipping that unit, competitors cannot cause food items to be skipped (Schoener 1974b). They can, however, affect the value of feeding in particular habitats and time periods, though ordinarily severe depletion must occur before it is optimal to no longer feed in a time period frequented by competitors. Time periods may be omitted if it is less costly (in terms of energy invested in foraging or of the probability of being eaten by a higher predator) to wait than to feed. Thus three factors may be responsible for the specialisations of adult M. merianae and M. menardi, relative to spiders which forage by day in the field layer: the investment made in web building, the relationship between spiders' sizes and

the severity of interspecific competition, and the influence of predation from higher predators.

The value of being a time or habitat specialist might increase with spider size if foraging is more costly in larger instars. However, Figure 5.1a shows that web diameter rises more-or-less linearly with cephalothorax length, and Figure 4.4 shows that body weight increases with the cube of cephalothorax length. Therefore, web area rises with weight$^{\frac{2}{3}}$ and web building is relatively less costly for adults than for smaller instars. On pages 65-66 I argue that intra- and interspecific competition is at its most severe among adult spiders and that population size is limited mainly by the density of adult females. It is probably for this reason that the adults of M. merianae and M. menardi are more habitat-specialised than juveniles are; but they would still gain from competing with M. mengei and M. segmentata for diurnal prey unless the risk of being eaten by diurnal visual predators is also a function of spider size. It may be economic for predators such as birds to search for and take only relatively large spiders (Bristowe 1941) with the result that size-dependent predation (Brooks 1968) enhances the value of being active only during the hours of darkness, which may be 100% of the time in caves.

Evidence for the influence of predation by visual hunters on Meta is circumstantial, but orb web spiders are potentially conspicuous targets for attack and adaptations for avoiding predation are evident in all three orb web families. For instance, Uloborus and some Araneidae build stabilimenta into their webs. These are silk structures which have no apparent mechanical function other than to guide the spider to the shortest exit from its web. Being more conspicuous than the spider itself, the stabilimentum acts as a

Figure 5.1 Meta orb web parameters. Sample sizes vary between 5 and 30 observations per instar. Vertical bars indicate the 80% confidence limits of the means.

5.1a Web diameter.

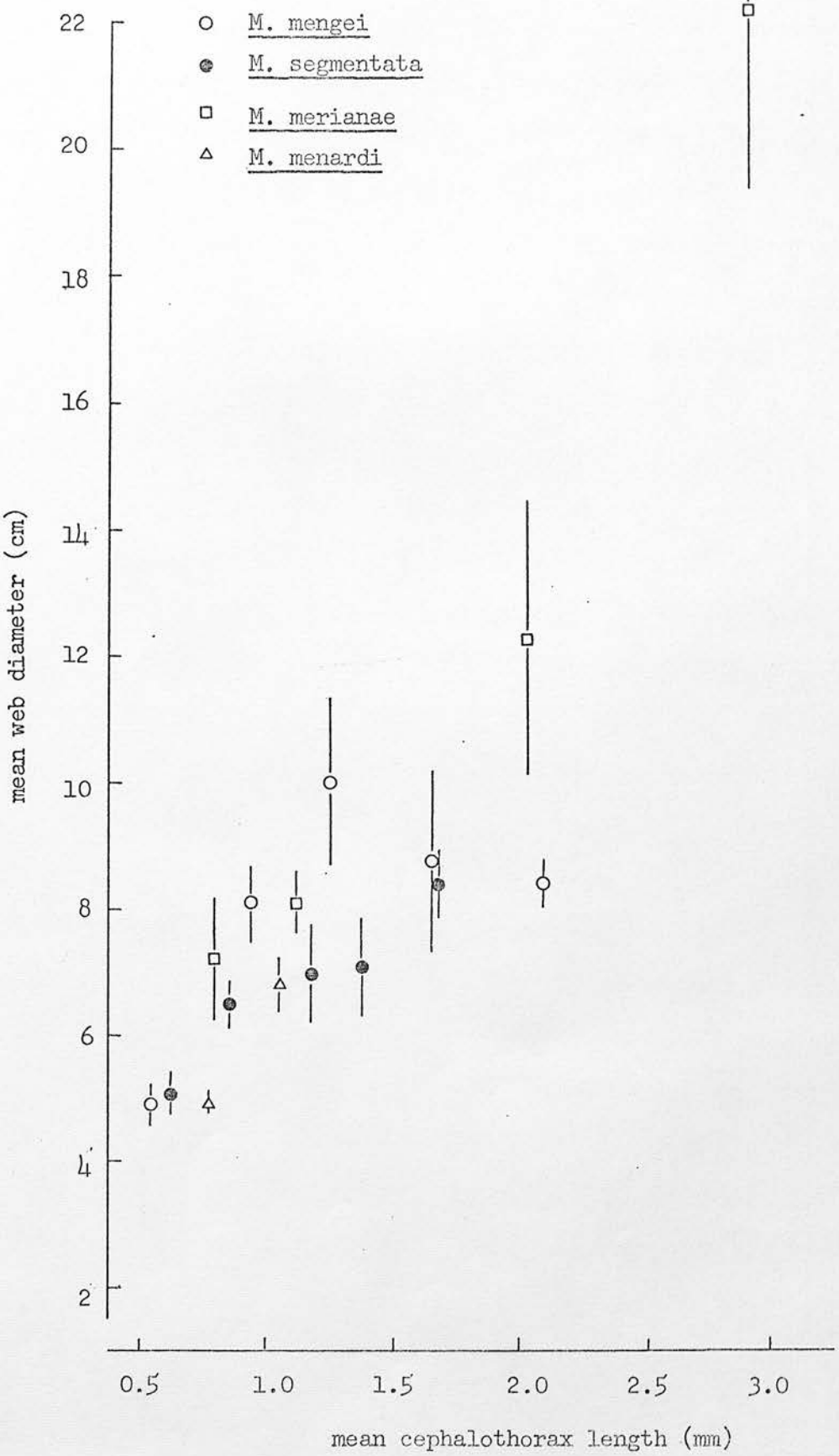
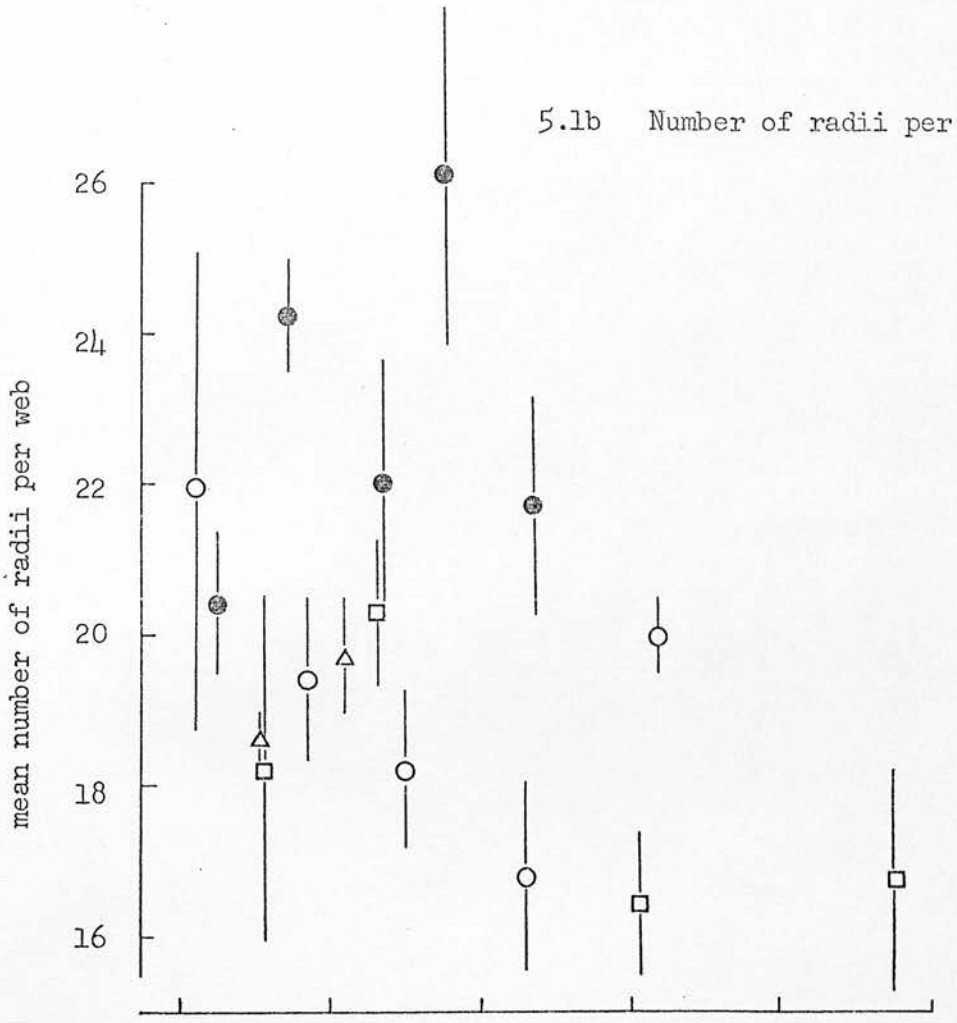


Figure 5.1 continued.



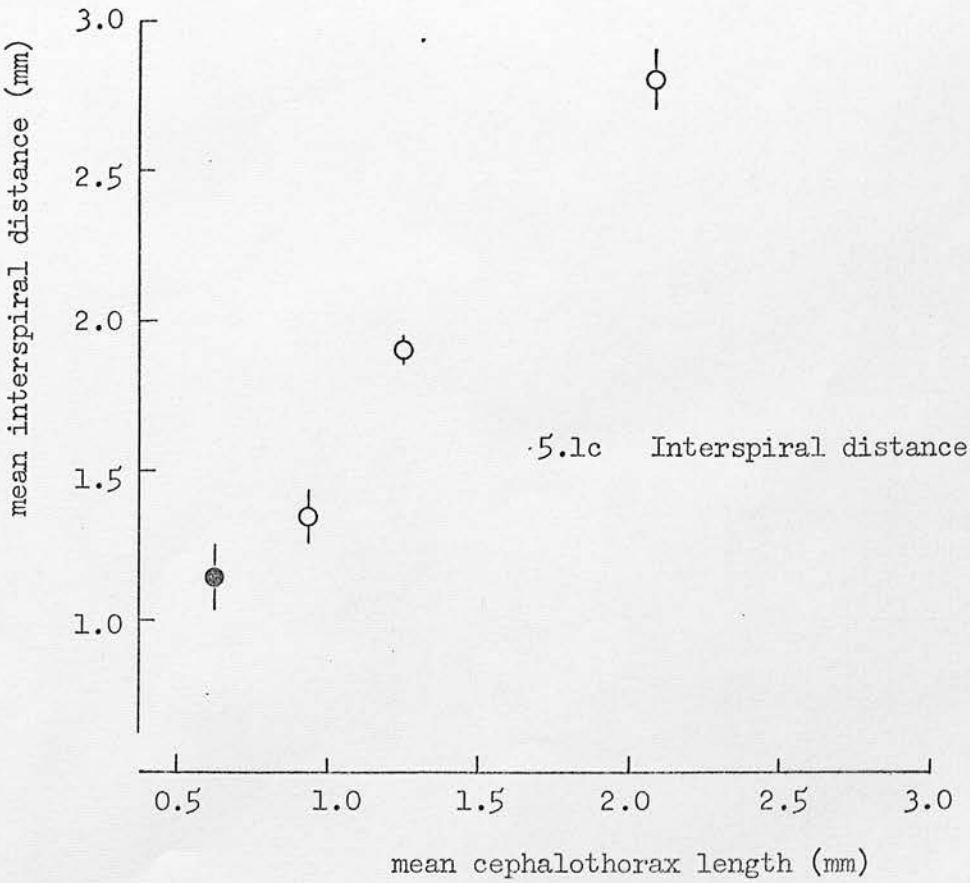
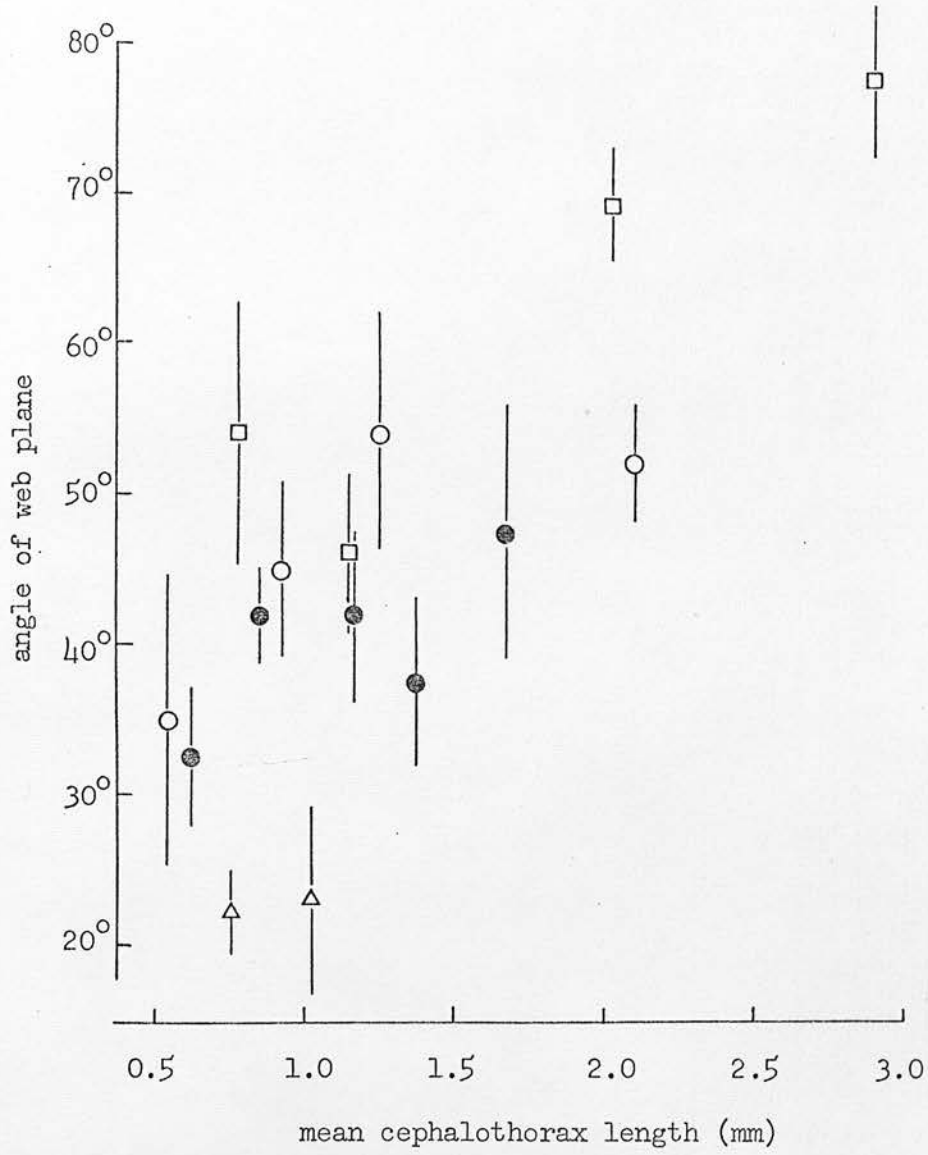


Figure 5.1 continued. 5.1d Web plane angles.



decoy which, when attacked, mis-directs the predator long enough for the spider to escape from its web safely (Eberhard 1973). When threatened by shadow or contact with its web, Araneus diadematus uses its web's elasticity to "buzz" itself in a rapid circular oscillation which completely blurrs the spider's outline. Tetragnatha extensa often spin their webs among grasses and reeds near water, situations which do not provide much surrounding vegetation cover. These spiders escape the attentions of predators by pressing their elongate bodies along web-supporting stems during the day and resume the head-down posture at the hubs of their webs in dim light. M. mengei and M. segmentata occupy slightly more sheltered web sites than Tetragnatha and these species rely mainly on cryptic colouration to escape detection. Their dorsal and ventral abdominal patterns effectively break up the spiders' outlines and blend well with heather and Luzula flowers. And in addition to falling from their webs and remaining motionless for a period when disturbed by shadows or vibrations, as most spider species do, both M. mengei and M. segmentata also adopt the strategy of keeping out of sight under leaves or against web supports, remaining in contact with the hubs of their webs via signal threads. This behaviour is most frequent in M. mengei in spring and early summer, when least shelter is provided by surrounding vegetation; and this is the very position adopted by adult M. merianae during daylight, except that in this species the signal thread has virtually become a permanent feature of the web and the spiders have ceased to respond to stimuli from their webs during the day. Adult M. merianae and M. menardi retreat into shade when disturbed by torchlight at night, but M. mengei and M. segmentata make no such response.

Juvenile M. merianae and M. menardi utilise the same diurnal

prey populations as similar-sized M. mengei and M. segmentata. But in M. merianae, the balance between the advantages of avoiding predators and competing with the field layer species is weighed more heavily in favour of predator avoidance than it is in M. menardi instars 2 and 3. Young M. merianae return to crevices and overhangs for a period of seclusion at each moult and disperse from there in the dark. Conversely, M. menardi's first feee-living moult takes place in the field layer and these juveniles become much more widely dispersed than young M. merianae. Compared with the number of potential web sites for adult M. merianae, adult M. menardi web sites are relatively scattered among many small, isolated microcaves in Argyll where this species passes through a phase of diurnal foraging and dispersion. Therefore, the potential disadvantages of being unable to find under-populated microcaves, before the young spiders are large enough to be under significant threat of predation by vertebrates, assume much greater importance in M. menardi than in M. merianae.

A second factor is possibly involved in the ecological difference between juvenile M. menardi and juvenile M. merianae. Young spiders probably face greater risks of being parasitised by Pompilinae than of being eaten by vertebrates, whereas the reverse may be true of older, larger instars. Pompilinae are fairly host-general (Bristowe 1941), and pompiline females compete with each other for productive spider hosts. However, young M. menardi may not be particularly suitable hosts for pompiline larvae because these spiders leave the field layer at the end of the 3rd instar. Pompilines which pupated in semi-isolated microcaves would have no immediate access to potential mates and juvenile spider hosts, and would consequently be at a competitive disadvantage to conspecifics

which developed and pupated in the field layer. Thus it might be to the advantage of pompiline females if they avoided laying their eggs on M. menardi juveniles, and it would certainly be to the advantage of M. menardi if they signalled their unsuitability as hosts. This could be the reason why M. menardi has evolved contrasty, conspicuous (to me) black and white patterns in the 2nd and 3rd instars. These spiders spin their webs at shallow angles (Figure 5.1d) and pompilines approaching from above are first confronted with two bold white "eye-spots" on the spider's black ventrum, and those approaching from below are confronted with two black eye-spots on the white background of the dorsum (Figure 1.1). The dorsal pattern may provide protection purely by breaking up the spider's outline, but although it is recognisably related to the patterns of the other Meta species, the contrasts of the pattern itself are sharp, as one would expect of a signal. Thus not only may M. menardi juveniles have more to gain by dispersing than M. merianae have, they may also have less to loose, being unsuitable as hosts to Pompilinae larvae.

The principle niche difference between M. mengei and M. segmentata is breeding season. Hutchinson (1959) observed that related sympatric species are often separated by linear size ratios of about 1.3. He suggested that these may represent specialisations for food item size. Breeding asynchrony establishes interspecific size differences between co-occuring instars of similar-sized hemimetabolous arthropods, and a one-instar time lag would set up a size ratio of approximately 1.3 between M. mengei and M. segmentata (growth factors: page 42).

Enders (1974) incorrectly assumed that Hutchinson's(1959) hypothesis is always valid. On the basis of spider sizes, seasons

and months of breeding, and microhabitats, he concluded from Luczak's (1963) data that "most (European) araneid spiders coexist by differences related to prey size: season and size of spider" , except where microhabitat differences obviate the need. However, the size ratios he quoted as evidence were calculated from adult female sizes, not from the ratio of co-occuring instars. Furthermore, within the spring and autumn seasonal groups, the smaller Araneus species reach maturity and breed before the larger species, so interspecific size differences must disappear for a time before re-emerging between co-occuring adults. Also, the size frequency distributions of the large autumn-breeding Araneus diadematus and the smaller spring-breeding A. cucurbitinus must overlap at times because both of these species are biennial in N. Europe (Toft 1976). Similarly, although the older M. mengei generation in Scotland initially has a three-instar lead over M. segmentata in June, this has shrunk to complete size-overlap by the 6th and 7th instars (Figures 4.5 and 4.6). Thus neither Enders' (1974) evidence nor the present data strongly support the interpretation that breeding season is a "dummy" variable for spider size, and hence prey size.

Later, Enders (1975) argued that large orb weavers generally segregate into separate microhabitats and that small orb weavers tend to occur in sets of different-sized species within microhabitats. His explanation for this was that: small Araneus invest relatively more energy on prey handling activities than large Araneus, and because they utilise the modal prey sizes (on Schoener and Janzen's (1968) prey size distribution) their prey species are relatively abundant; therefore small Araneus are pursuers (MacArthur and Levins 1964) which specialise on narrow prey size ranges, relative to large Araneus, which are searchers and unable to coexist by prey

size differences. However, this arguement was largely based on Enders' (1974) previous interpretation, in which he accepted without question that body size is adequate as a niche difference.

In contrast to Enders (1974, 1975), Wilson's (1975) theoretical treatment of predator-prey size relationships predicts that in spider-sized insectivores feeding in the 1 - 10mm prey-size range, consumer size may never be adequate as a niche difference. He concluded that only the top predators in the system, birds and lizards in the case of insectivores, may coexist by differences related to prey size. The model is based on observation and its distinguishing feature is that it takes into account the asymmetry that exists in the feeding relations of different-sized predators: large animals eat things which are unavailable to smaller competitors, but the reverse is much less true. He rejected the assumption, implicit in Enders' (1974, 1975) reasoning, that a predator's success of capture is symmetrically distributed around one mean prey size. Instead, the model assumes that capture success rises from a lower prey size limit, which may largely be determined by perceptual parameters, to a broad plateau of peak success, before falling again towards the upper size limit of the predator's prey handling abilities.

Observation (see Footnote) suggests that these conditions

Footnote. Here I summarise my conclusions from field observations and examination of over 170 prey items of Meta collected by the methods outlined on pages 40 and 41. Unfortunately, because these spiders chew their prey, too few of these items could be measured accurately or identified to family for quantitative presentation. Most instars of M. mengei were represented in the data and this species is used here as as example.

are fully met in Meta. The smallest instars mainly catch very small Diptera, small Chironomidae, Ceratopogonidae, and Cecidomyidae for example, which are infrequently larger than 2mm long. Prey size variance increases with spider size. Fourth and 5th instar M. mengei can subdue prey up to 5 - 6mm; Anisopodidae and Mycetophilidae for example. The prey of adult M. mengei overlap with the smallest prey sizes of 2nd instars, but adults can also subdue heavily built fies of up to about 1cm, such as Syrphidae and Muscidae. Wilson's predator size A starts feeding at prey sizes of 1mm, reaches peak success at about 2.5mm, and catches prey of 6 - 10mm with increasing difficulty. This is probably a fair description of the prey-capture abilities of adult female M. mengei and M. segmentata, although 10mm is not absolutely the upper prey size limit of these spiders, larger Nematocera also being caught occasionally.

Ironically, I designed the prey sampling program with a view to demonstrating a symmetrical relationship between spider size and prey size (page 33): but although the interspiral distances of orb webs are linearly related to the cephalothorax lengths of the builders (Figure 5.1c), webs do not appear to act in a symmetrically selective way. Small prey rarely pass between the sticky spirals of adult female M. mengeis' webs without touching and being held at least temporarily. However, silk thickness is related to spider size also, and there is probably a correlation between silk thickness and the threshold of prey momentum necessary for the transmission of stimuli from the point of impact to the spider's sense organs. Nevertheless, adults frequently respond to the smallest size of prey caught by 2nd instars, and once perceived, no small item is rejected. Interspiral distance increases with instar size not as a means of skipping small prey items, but as a means of raising the upper size

limit of prey which will be held long enough for the spider to attack and, if necessary, bind with additional silk. Small items stick in adults' webs, but large items bounce off small spiders' webs. Adults have the strength and weight to tackle large prey, but small instars retreat to respectable distances when large prey strike their webs. The relationship between spider size and interspiral distance thus reflects the balance between the potential gains from catching large prey (more digestible tissue per unit of energy invested in prey handling) and the potential costs of intercepting prey which are too large to subdue (energy lost in fruitless struggles and damaged webs).

Wilson (1975) first determined the outcome of competition between different-sized predators when resources are equally available in all prey size classes, an unrealistic situation. Here the model predicts "limiting similarity", where a resident species excludes slightly smaller competitors, although larger and comparatively small (< 0.5 resident size) competitors may invade the microhabitat. When a log-normal prey size distribution (Schoener and Janzen 1968) is applied to the data, however, the outcome of competition depends upon where the competitors lie on the resource utilisation spectrum. Sets of large predators (eg. birds) may coexist by prey size differences, but in the case of predator A (spider-sized), the limiting similarity becomes a "competitive gradient" and the larger species always has the competitive advantage over the smaller species. The reason for this lies with the feeding asymmetry: in the upper range of the larger predator's capture success curve, resources (near the mode of the prey size distribution) are relatively abundant, and these are unavailable to the smaller competitor; so the larger predator can tolerate resource levels at which the smaller species can no longer

reproduce. The larger species is said to have the "K' advantage" over the smaller competitor.

Thus the theoretical model pedicts that the majority of spider species cannot coexist by prey size differences alone, though it is quite possible that large Araneidae, which may be the top predators in place of lizards or birds in some situations, can segregate along the prey size niche dimension. Enders (1975) argued the converse: that small Araneus can partition prey size and that large Araneus cannot. However, Wilson's (1975) model is much more convincing than Enders' (1975) because it successfully unifies results from both observational and experimental spider ecology. Having the K' advantage does not only mean that a predator can tolerate lower resource levels than smaller competitors, but also that that predator is more "efficient" at depleting resources. It follows that large predators deplete each others resources "better" than small predators do; and that the density-dependent effects of intraspecific competition will be more evident in large spider instars than in smaller conspecific instars.

Two independent examples illustrate this. Riechert (1974) showed that the funnel webs of adult Agelenopsis aperta (Agelenidae) are more regularly spaced than are the webs of juveniles. The area of occupation increases with spider size, and she concluded that territory size, and hence the degree of regularity of web spacing, is related to securing food supply. In other words, as the spiders become more efficient predators, so the space each requires to avoid depleted areas created by conspecifics increases; and in finite space, the system becomes more ordered and the negative influence of intraspecific competition becomes more severe. Essentially, this is Wise's (1975) conclusion: that the interaction between spider

density and food supply is a function of the ages of the spiders. By artificially adjusting population densities, Wise (1975) showed that the growth rates of small juvenile Linyphia marginata (Linyphiidae) are virtually independent of population density, even though feeding rates limit growth rates (they grow faster when supplied with food manually): but in older instars, growth rates become progressively more dependent on population densities; and in adult females, competition for food limits fecundity.

Food supply primarily affects the growth rates of juvenile spiders, though it also influences size after the next moult (Turnbull 1965). Feeding rate during growth may influence fecundity indirectly, via its influence on size in the final instar (clutch size covaries over taxa with adult female length (Toft 1976), and clutch size and eggsac weight covary with adult female cephalothorax length and weight in M. segmentata (Geyer 1971)): but the proportion of adult female weight which is channelled into reproduction largely depends on feeding rate in the final instar. Since large spiders are more efficient predators and also more sensitive to competition than small spiders, therefore, population size may principally depend upon the negative influence of adult female density on adult female fecundity. This applies equally to populations of conspecific adult females and to mixed populations of congeneric adult females, so long as the spiders make their livings in much the same way, and consequently compete. It follows that any strategy which aleviates competition between adult females will be favoured, and two sister species which overlap spatially, due to constraints imposed by other competitors or predators for instance, will diverge along the breeding season niche dimension. Breeding asynchrony is a stable strategy because divergence permits a rise in the

instantaneous rates of increase in both populations, until the population sizes are once again limited by food supply and the densities of adult females. This is the significance of the breeding season niche difference between M. mengei and M. segmentata.

M. mengei and M. segmentata become vertically stratified in autumn (page 47), primarily because adult female M. segmentata select tall stems of vegetation to spin their webs on. Vertical height in the field layer is the primary niche difference between some North American Araneus species (Enders 1974), but by late September, the webs of adult female M. segmentata are often no more than 5cm in diameter, possibly because egg production has priority over silk production, and there is no indication that vertical stratification aleviates interspecific competition between these siblings. I suggested on page 56 that M. merianae and M. menardi breed when their respective microhabitats are most productive: the same is true of M. mengei. In fact, adult female M. mengei probably retire from foraging in late September, and do not become active again until the first prolonged warm period in April, because it is less costly to skip that time period than it is to build webs. Female M. segmentata, on the other hand, become adult in August and face a limit on the time available for mating and producing eggs before resources become very scarce in late autumn. By occupying web sites in tall vegetation, however, adult females may significantly reduce the rate of lateral dispersion of adult males in the field layer, and effectively "trap" males in their proximities for longer than would otherwise be the case, hence hastening courtship and copulation.

Adult female M. mengei which are unprepared to mate with courting males run the risk of being robbed. Sexual interactions

in this genus are usually initiated by prey striking the female's web (Bristowe 1958), and during the bout of behaviour which follows, the male M. mengei tickles the female's cephalothorax and abdomen with the hairs of his first leg metatarsi and tarsi. This presumably stimulates the female, but since males compete with each other for potentially fecund females, by doing this he may also measure how gravid she is, abandoning or perservering with courtship accordingly. The female may accept him, but she usually either responds aggressively, in which case he retreats, or she may herself retreat from the web, in which case he may assume the head-down foraging posture at the hub of her web, finishing the female's meal in the meantime. Thus by robbing the female, the male feeds at her expense. This is in his interests, because he obtains energy with which to continue his search for virgin females, and it is in the interests of the female with whom he eventually mates, because a competitor has been robbed and because without food the male may not have reached her. Although I speculate on the basis of few observations, it seems that female M. segmentata accept males more readily, and that male M. segmentata rob females less frequently than male M. mengei (if ever). However, this is to be expected, because being (relative to male M. mengei) "cornered" in the proximity of the female at the top of a tall stem, he is more likely to mate with that female, and it would not be in his interests to rob her.

In conclusion, these are the main points arising from the discussion in this chapter. Large instars are more efficient predators and more sensitive to the negative influence of competition with similar-sized instars than smaller conspecifics are. Consequently, population size is mainly limited by food supply and

adult female density. For this reason, the breeding activities of the four Meta species have diverged along the seasonal time and horizontal space niche dimensions, although juveniles overlap spatially and temporally. Large spider instars are more vulnerable to attack by visual predators than juvenile conspecifics are, and predator avoidance has influenced the evolution of nocturnal activity in adult M. merianae and M. menardi instars 4 - 7. Predator avoidance has also sharpened the habitat differences between the species: adult M. merianae shelter from diurnal predators in crevices and under overhangs, and M. menardi is normally confined to caves, where no predator relies on hunting by sight. Juvenile M. merianae and 2nd and 3rd instar M. menardi forage by day in the field layer in order to utilise the small diurnal Diptera which are normally the food of M. mengei and M. segmentata. However, it is to the advantage of 2nd and 3rd instar M. menardi to disperse widely and colonise underpopulated microcaves, whereas it is to the advantage of young M. merianae to stay in shaded parts of the field layer and to return to crevices to moult, in order to avoid predators.

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Appendix 1.

(a) Horizontal starch gel systems.

Tris-Citrate pH 6.2:	Tris (Sigma T-1378)	0.223M	27g
	Citrate (Analar 10081)	0.085M	18.1g
	in 1 litre distilled H_2O		

-pH adjusted with M NaOH. Electrode buffer as above. Gel buffer: 17.5 stock solution in 500ml gel; 10% Electrostarch.

Tris-EDTA-Borate:	Tris	0.9M	109g
	EDTA (Sigma ED2SS)	0.02M	7.6g
	Boric acid (BDH 27410)	0.5M	309g
	in 1 litre distilled H_2O		

-pH adjusted with M HCl / NaOH. Cathode: 1/5 stock solution. Anode: 1/7 stock solution. Gel buffer: 25ml in 500ml gel; 12% Electrostarch.

(b) The following assays were made on both these gel systems: lactate dehydrogenase (LDH), malate dehydrogenase (MDH), xanthine dehydrogenase, isocitrate dehydrogenase, glucose-6-phosphate dehydrogenase, sorbitol dehydrogenase, 6-phosphogluconic acid dehydrogenase, α-glycerophosphate dehydrogenase (α-GPDH), alcohol dehydrogenase, phosphoglucose isomerase (PGI), phosphoglucose mutase, glutamate-oxaloacetate transaminase (GOT), leucine amino peptidase, peptidases, acid and alkaline phosphatases, and non-specific esterases.

(c) The folowing solutions were used for routinely staining esterases, LDH, MDH, GOT, PGI, α-GPDH (Sigma chemicals):

Esterases: 25mg α-naphthyl proprionate N-0376, dissolved in 1ml 10% acetone

25mg Fast Garnet F-0875

50ml 0.2M Tris-HCl pH 8.0

GOT: 60mg L-aspartate A-9256

10mg α-ketoglutarate K-1750

5mg pyridoxal-5-phosphate P-9255

50ml Tris-HCl pH 8.0

LDH: 5ml Na D-L lactate L-7004

25mg NAD^+ N-7004

15mg NBT N-6876

2mg PMS P-9625

45ml Tris-HCl pH 8.0

MDH: 5ml M Na-L-malate M-1125

10mg NAD^+

10mg MTT M-2128

2mg PMS

45ml Tris-HCl pH 8.0

α -GPDH: 40mg α-DL glycerophosphate G-6126

15mg NAD^+

10mg NBT

2mg PMS

50ml Tris-HCl pH 8.0

PGI: 15mg Na-D-fructose-6-phosphate F-3627

4 l glucose-6-PDH activity G-7750

0.8mg $NADP^+$ N-0505

15mg MTT

2mg PMS

30ml Tris-HCl pH 8.0

The immediate source of these buffer and stain recipes was Margaret Bathgate.

Appendix 2.

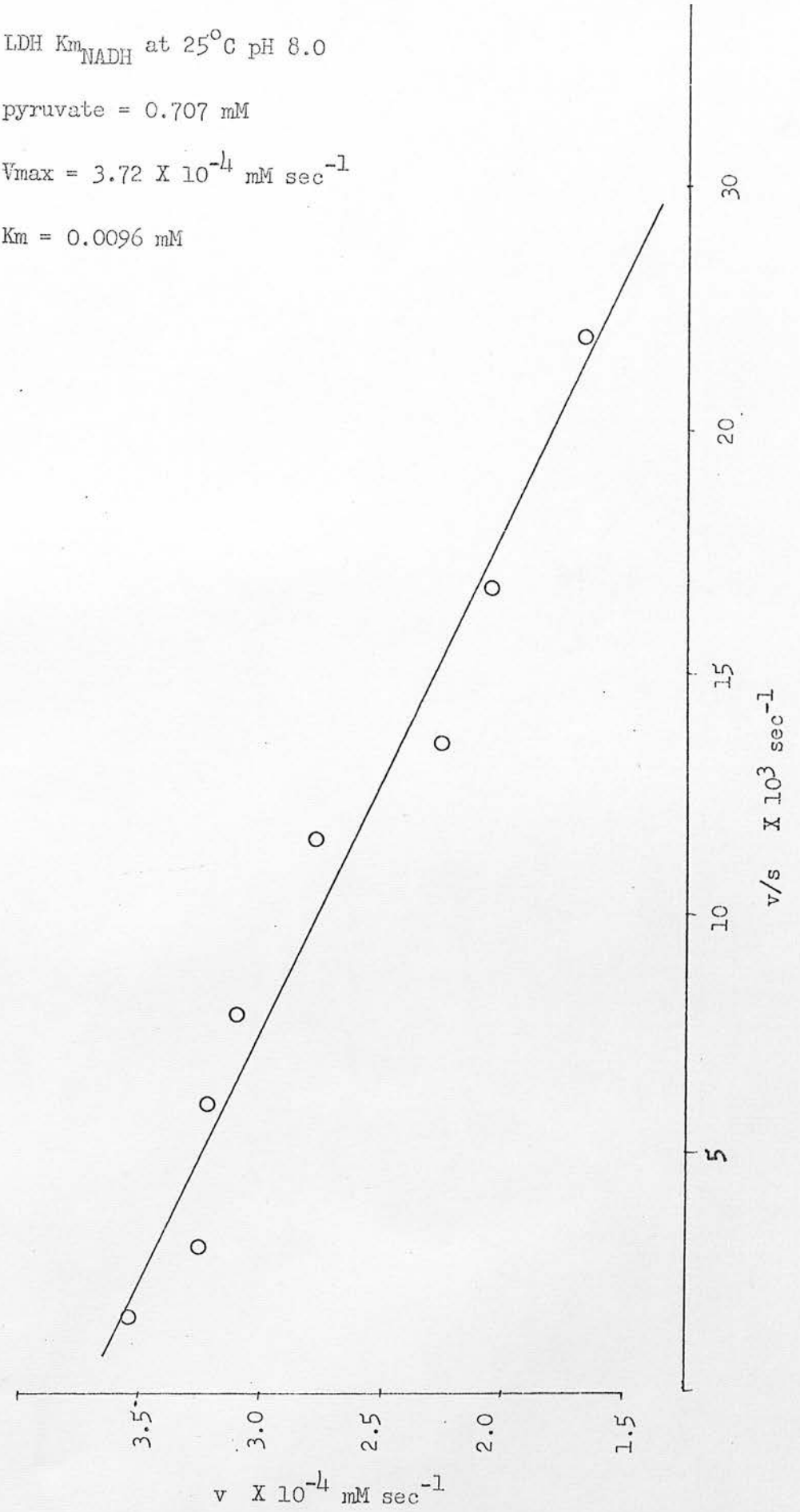
Two forms of graphical analyses were used to obtain Km and Vmax: linear regression of initial reaction velocity (v.) on v / s (substrate concentration), and 1/v on 1/s (Lineweaver-Burke plot). In the former, the intercept on the ordinate equals Vmax, and the slope of the line equals -Km. In the latter, the intercept on the ordinate equals 1/Vmax, and the intercept on the abscissa equals 1/Km.

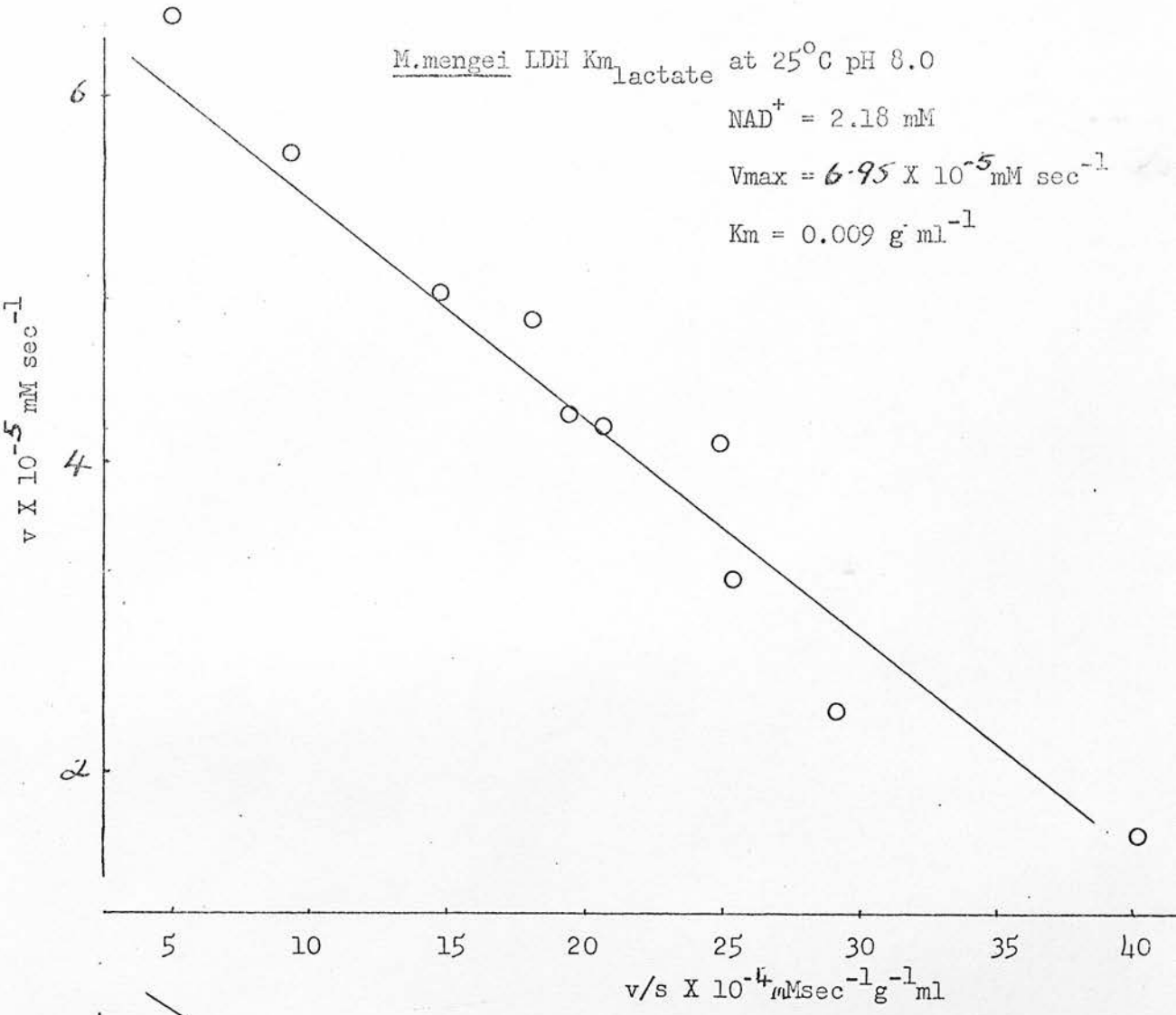
M.mengei LDH Km_{NADH} at 25°C pH 8.0

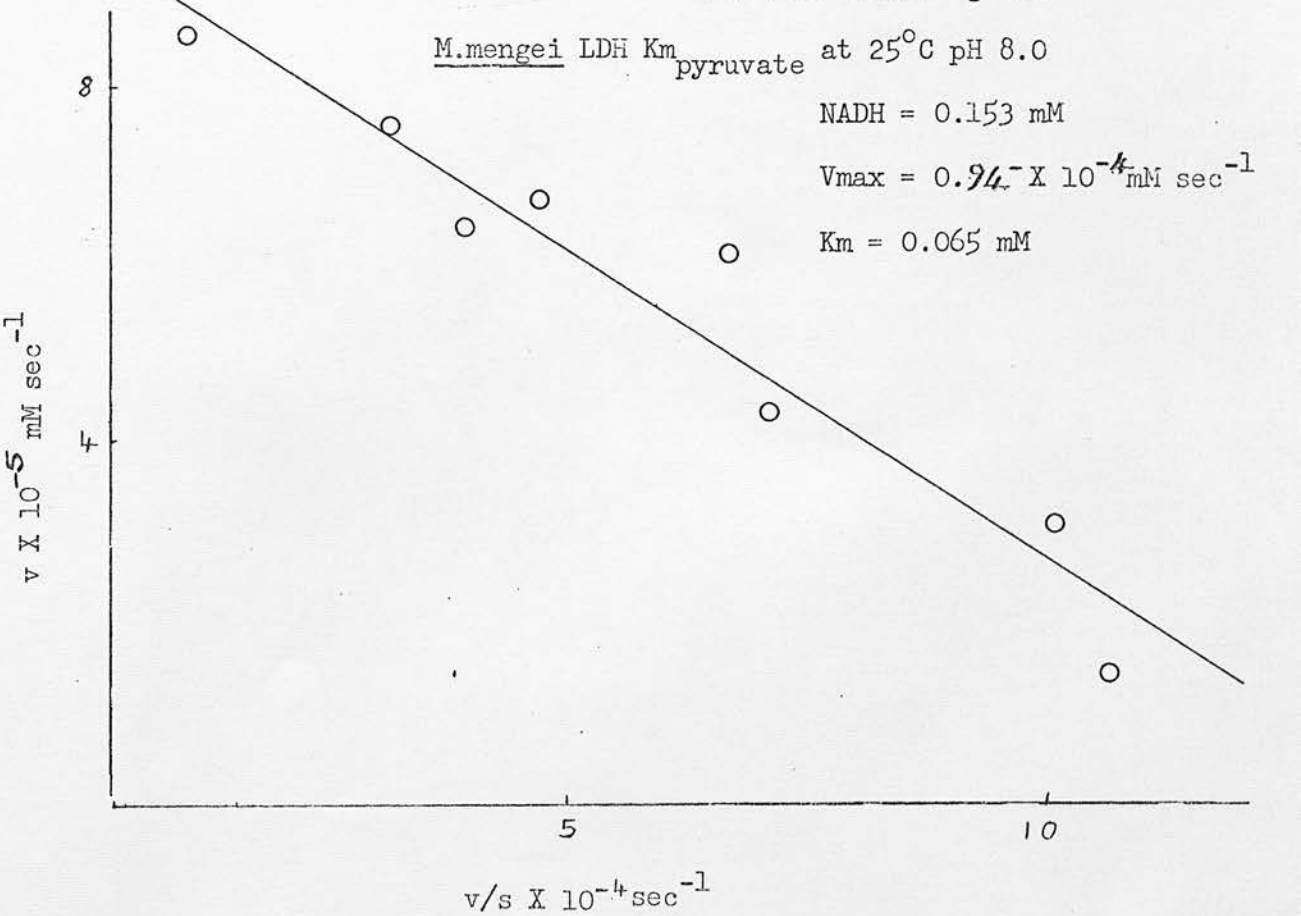
pyruvate = 0.707 mM

Vmax = 3.72 X 10^{-4} mM sec^{-1}

Km = 0.0096 mM




M.mengei LDH Km lactate at 25°C pH 8.0
NAD+ = 2.18 mM
Vmax = 6·95 X 10-5 mM sec-1
Km = 0.009 g ml-1
v X 10-5 mM sec-1
6
4
2
5
10
15
20
25
30
35
40
v/s X 10-4 mMsec-1g-1ml

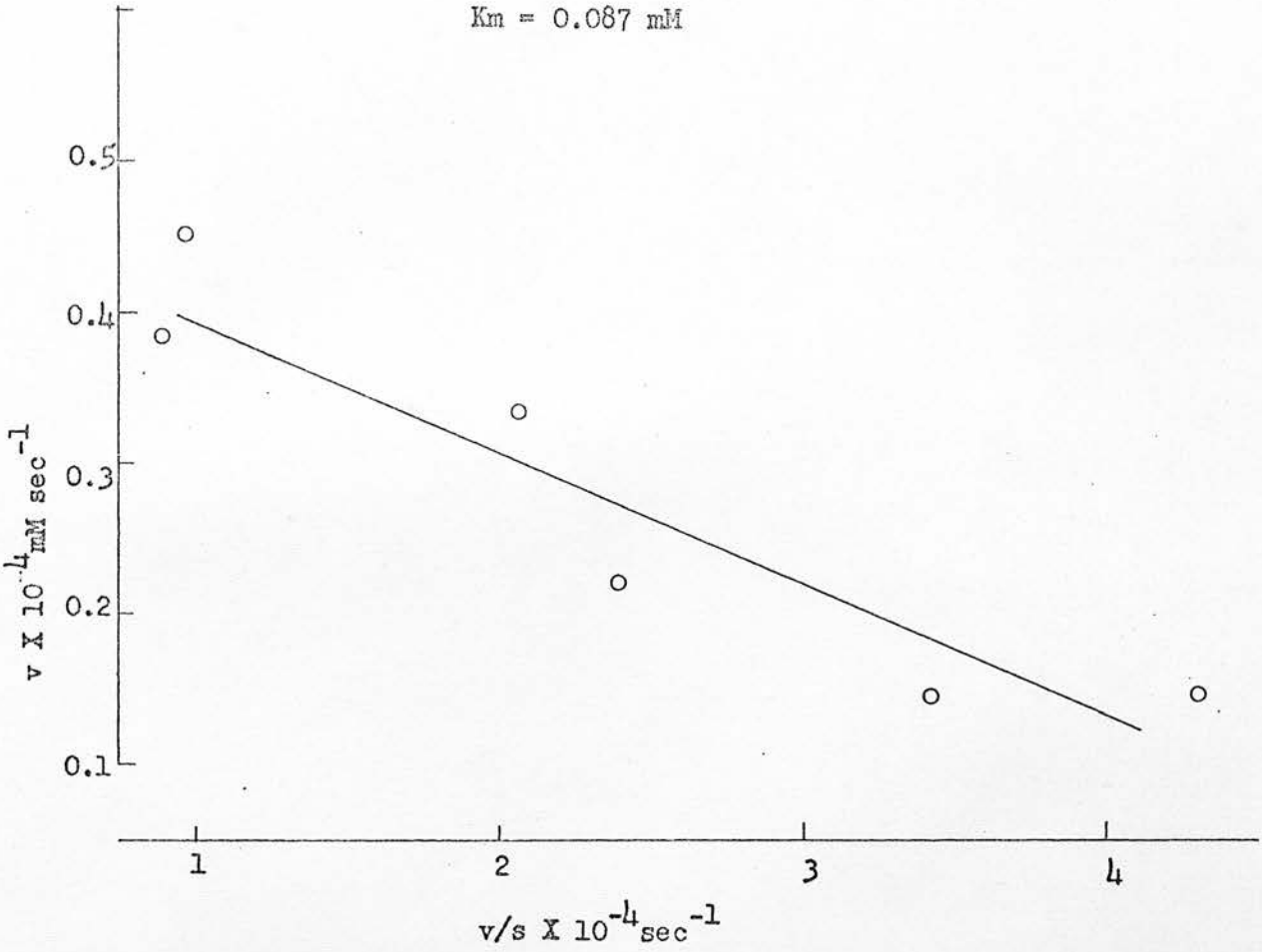

M.mengei LDH Km pyruvate at 25°C pH 8.0
NADH = 0.153 mM
Vmax = 0.94 X 10-4 mM sec-1
Km = 0.065 mM
v X 10-5 mM sec-1
8
4
5
10
v/s X 10-4 sec-1

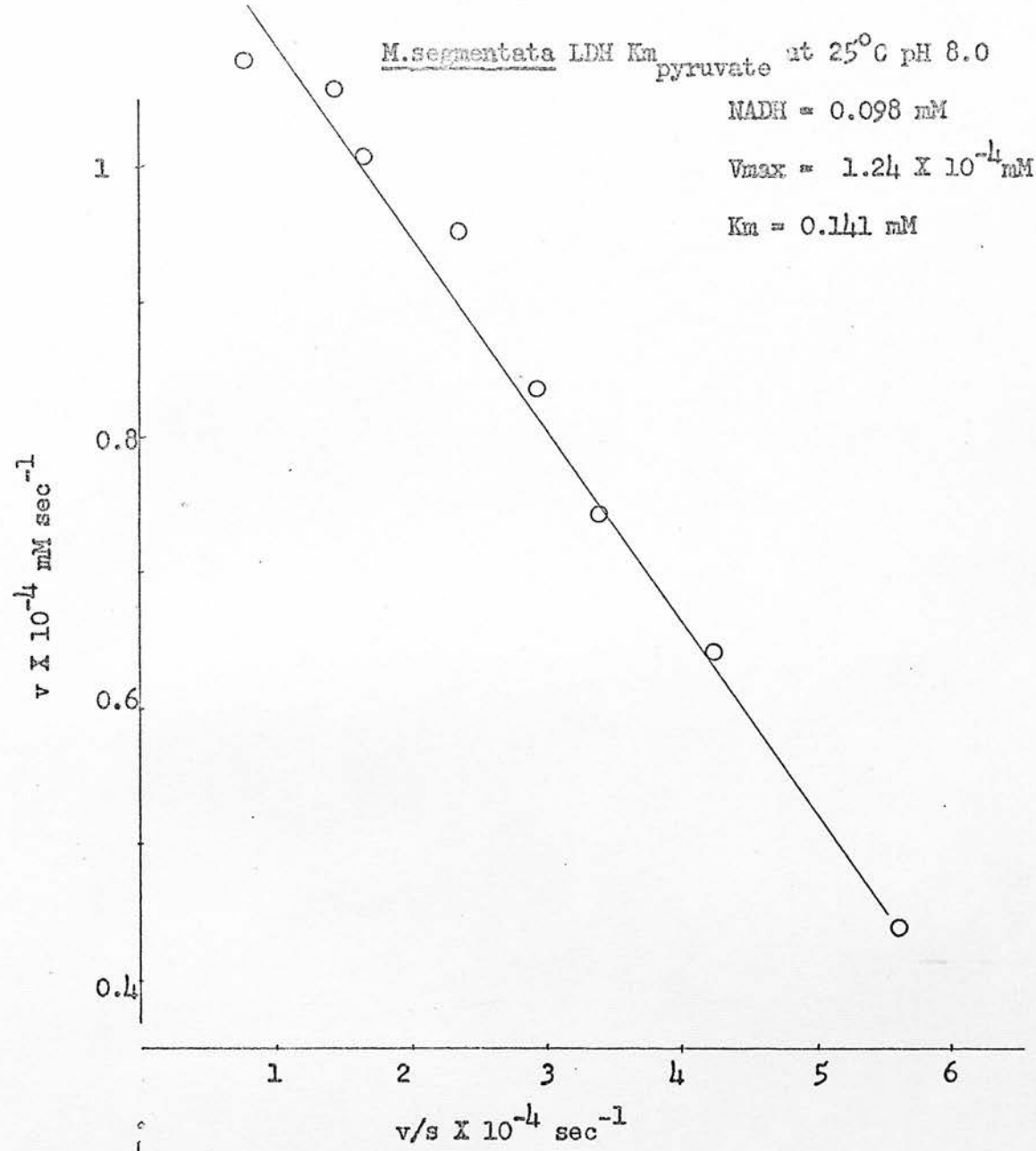
M. mengei LDH Km_{NAD^+} at 25°C pH 8.0

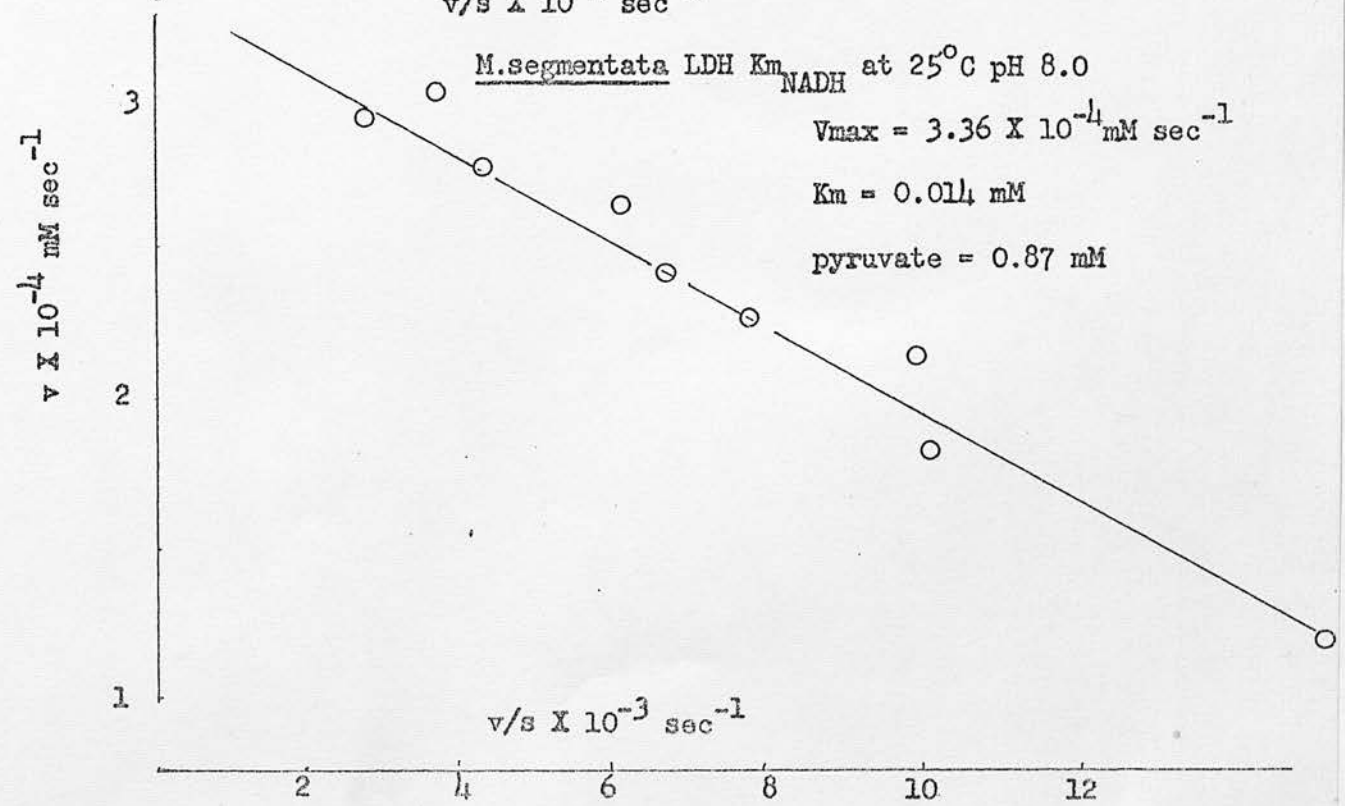
lactate = 0.09g ml^{-1}

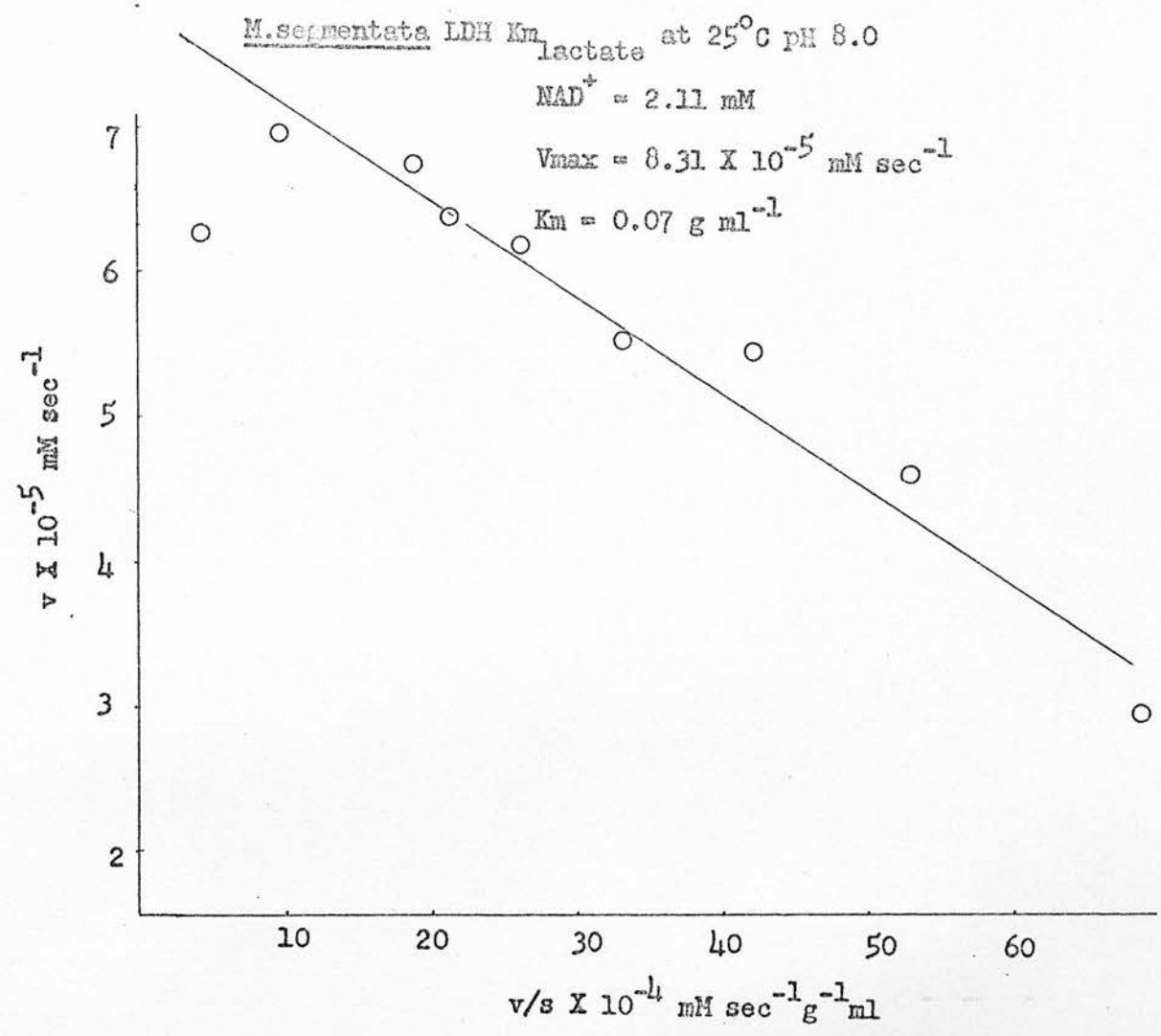
Vmax = 0.47 X 10^{-4}mM sec^{-1}

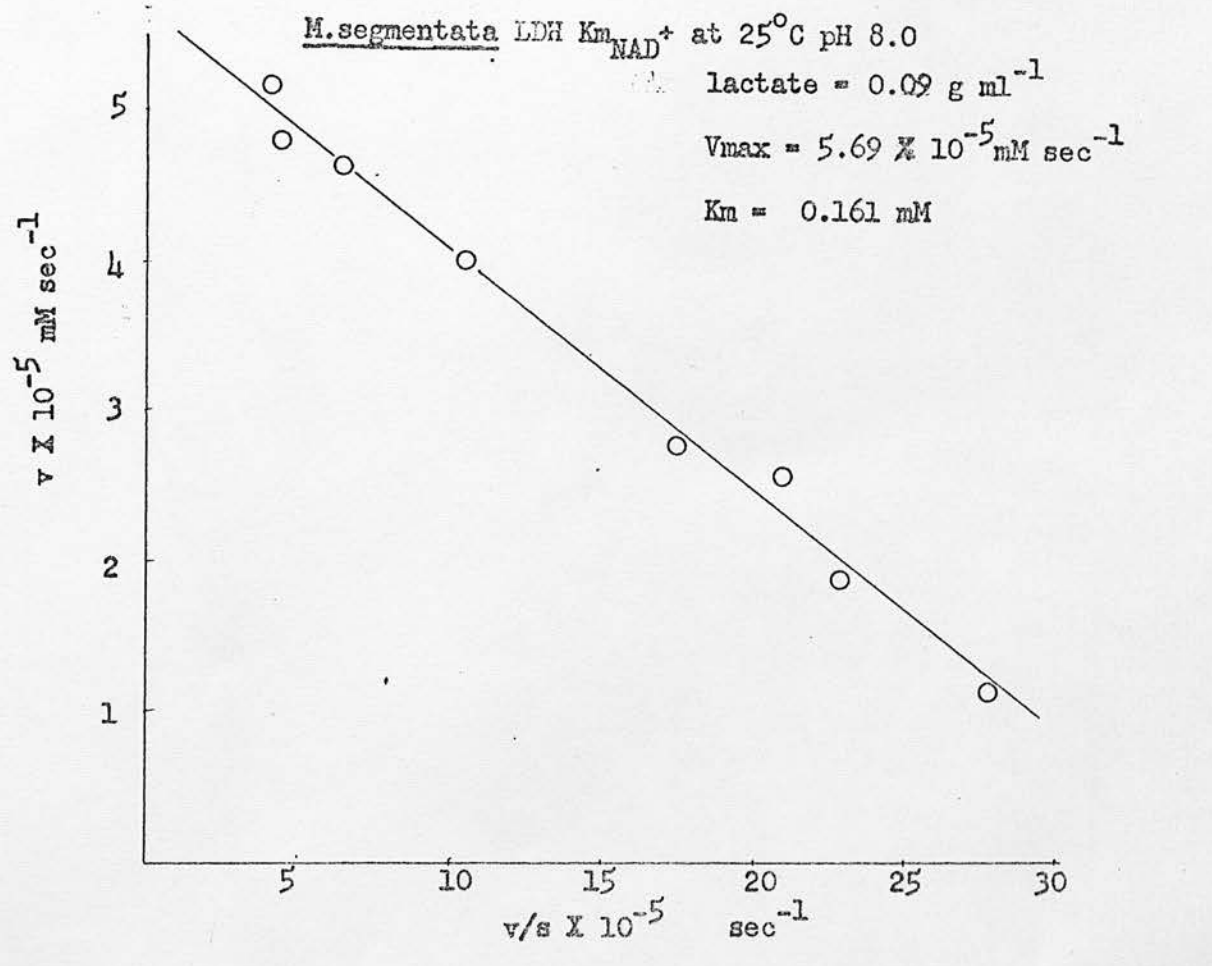
Km = 0.087 mM

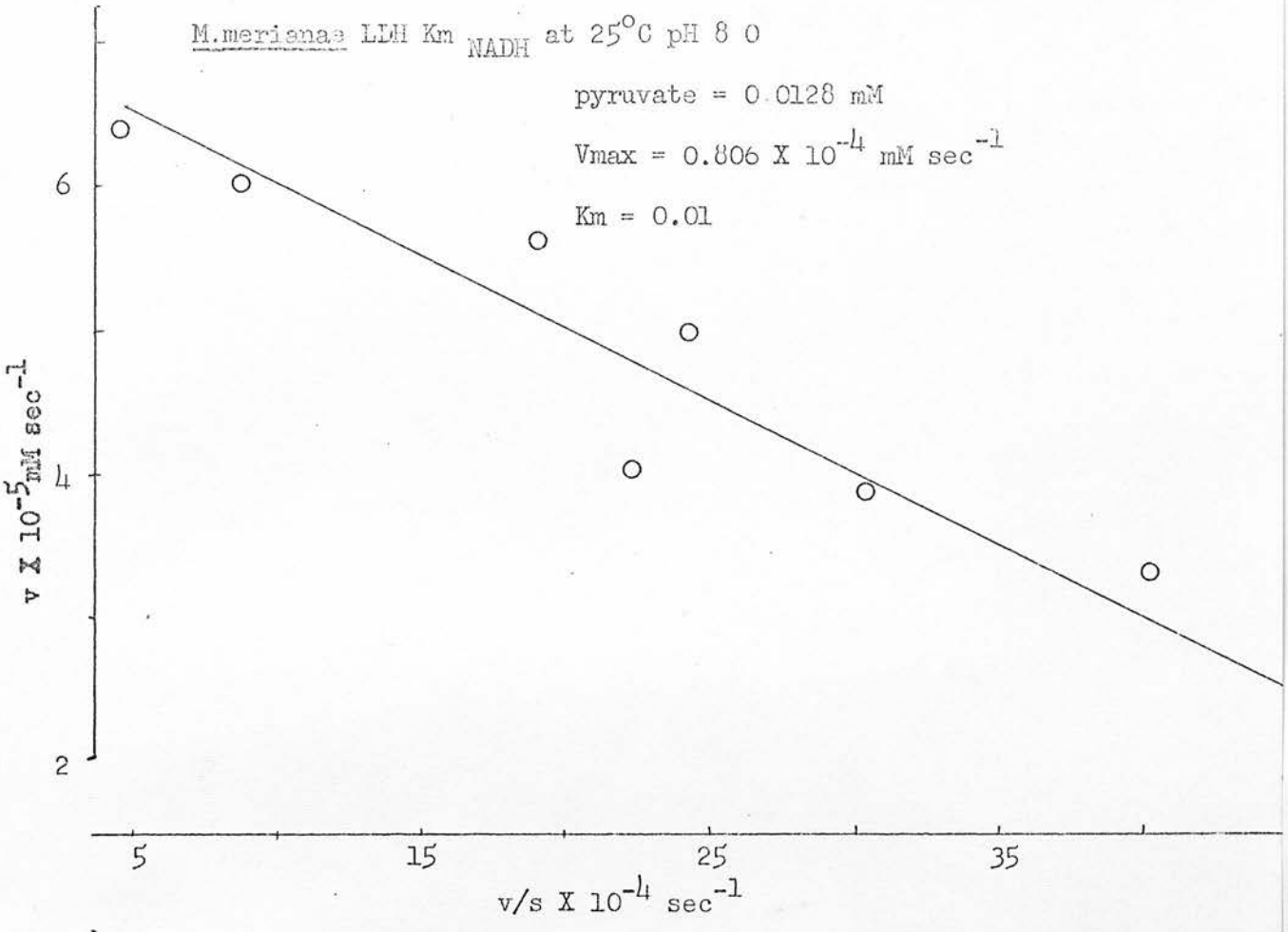


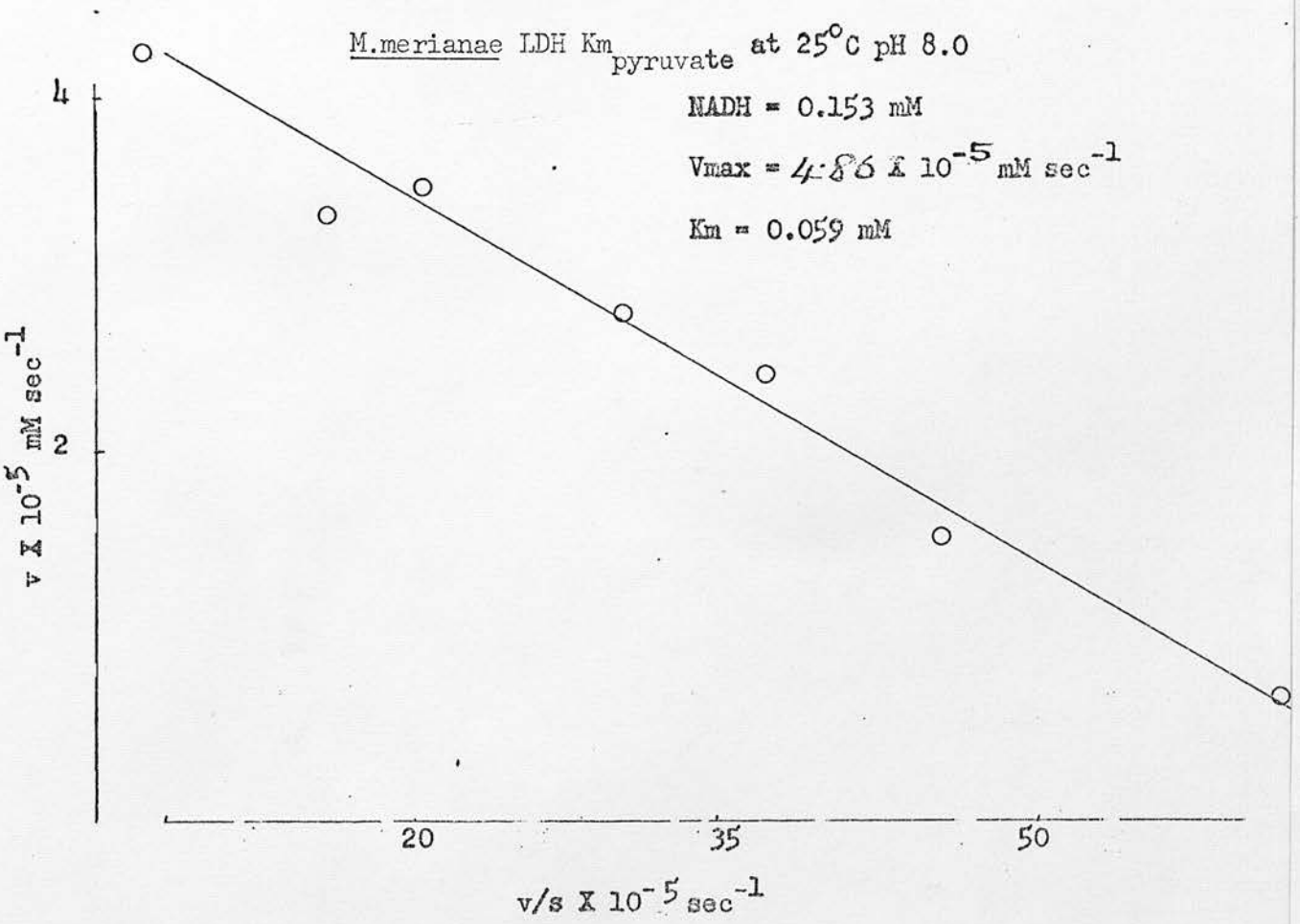

M.segmentata LDH Km pyruvate at 25°C pH 8.0
NADH = 0.098 mM
Vmax = 1.24 X 10-4 mM sec-1
Km = 0.141 mM
v X 10-4 mM sec-1
1
0.8
0.6
0.4
1
2
3
4
5
6
v/s X 10-4 sec-1

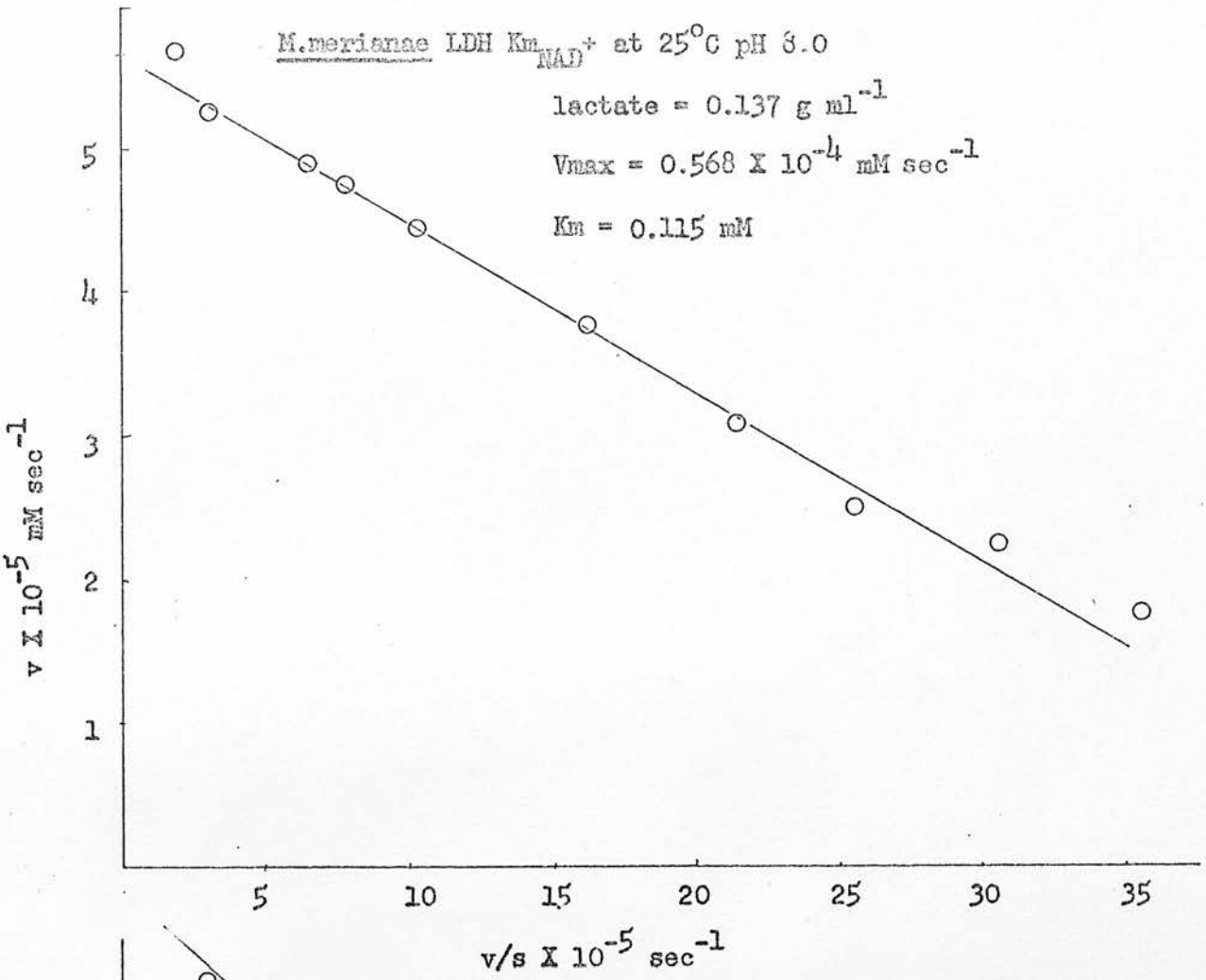

M.segmentata LDH Km NADH at 25°C pH 8.0
Vmax = 3.36 X 10-4 mM sec-1
Km = 0.014 mM
pyruvate = 0.87 mM
v X 10-4 mM sec-1
3
2
1
2
4
6
8
10
12
v/s X 10-3 sec-1

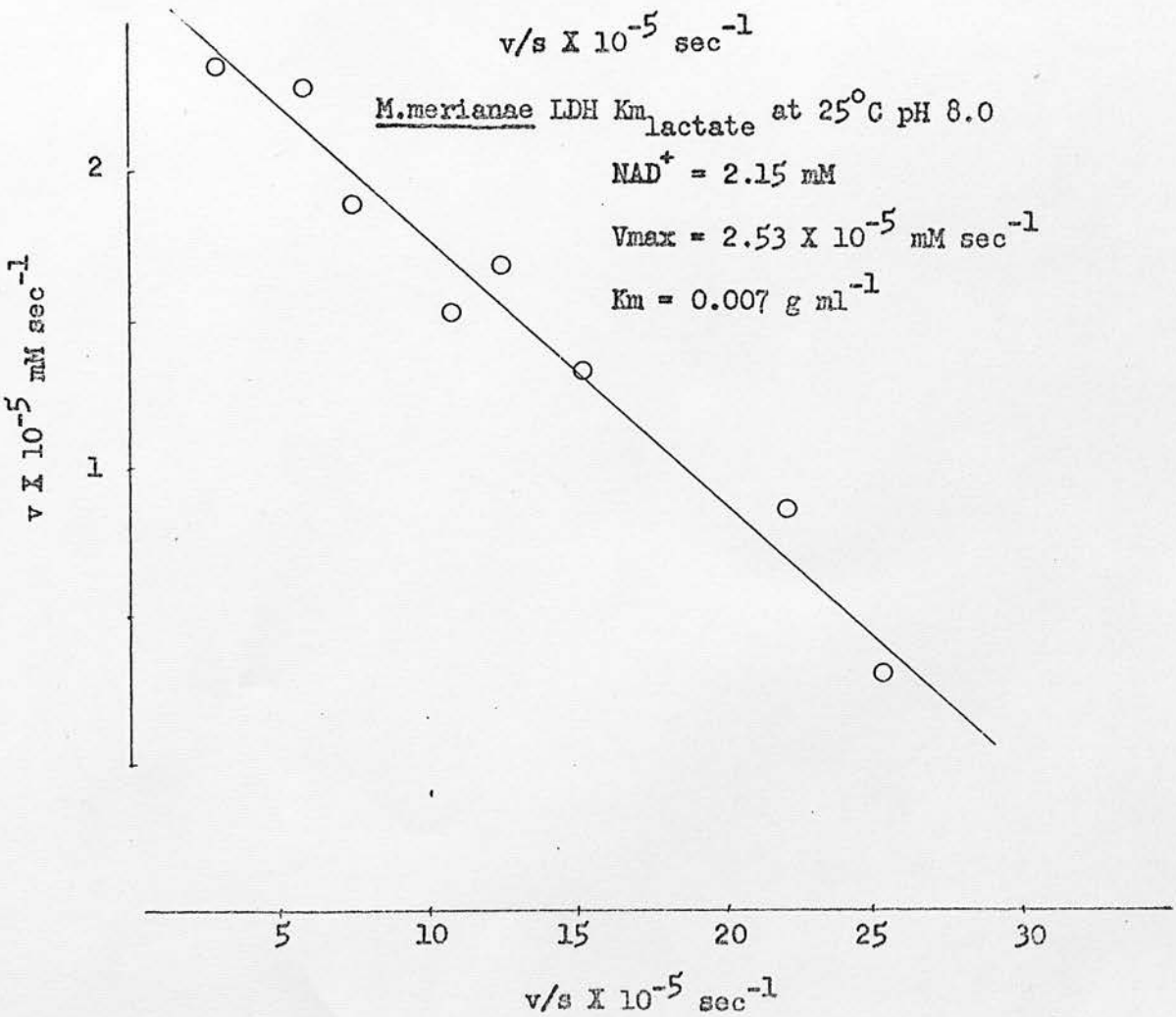

M.segmentata LDH Km lactate at 25°C pH 8.0
NAD+ = 2.11 mM
Vmax = 8.31 X 10-5 mM sec-1
Km = 0.07 g ml-1
v X 10-5 mM sec-1
v/s X 10-4 mM sec-1 g-1 ml
2
3
4
5
6
7
10
20
30
40
50
60

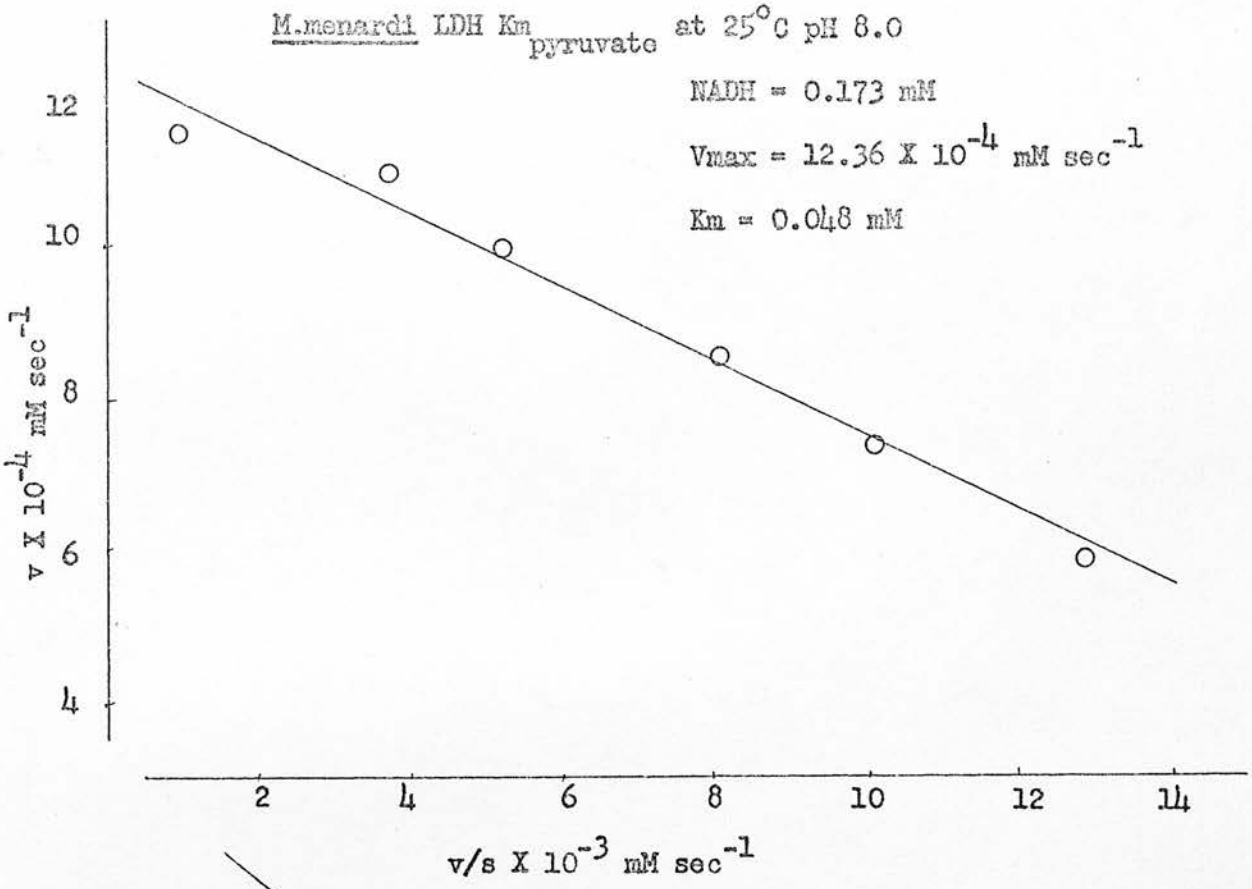

M.segmentata LDH Km NAD+ at 25°C pH 8.0
lactate = 0.09 g ml-1
Vmax = 5.69 X 10-5 mM sec-1
Km = 0.161 mM
v X 10-5 mM sec-1
v/s X 10-5 sec-1
1
2
3
4
5
5
10
15
20
25
30

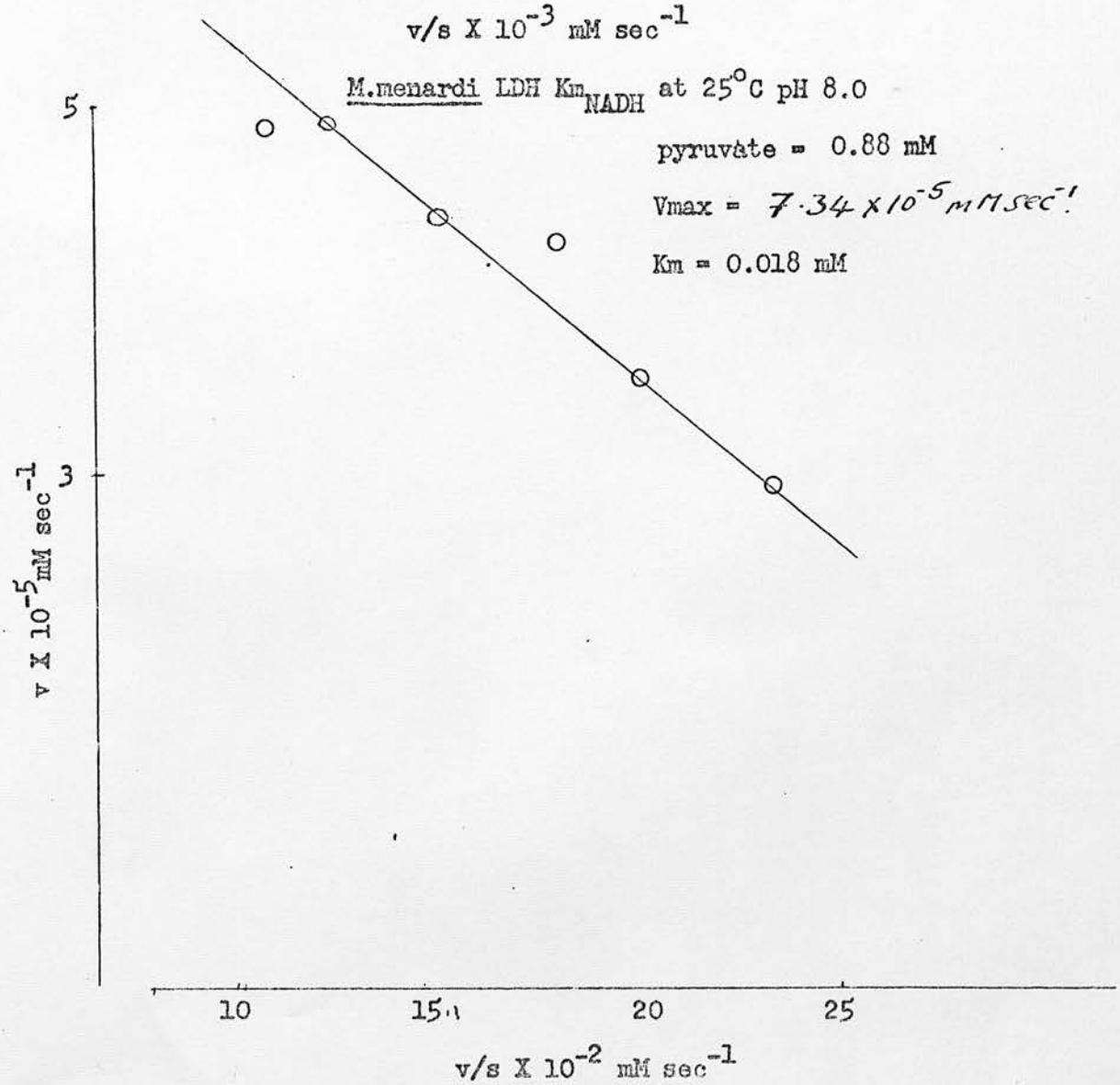

M.merianae LLH Km NADH at 25°C pH 8 0
pyruvate = 0.0128 mM
Vmax = 0.806 X 10-4 mM sec-1
Km = 0.01
v X 10-5 mM sec-1
v/s X 10-4 sec-1
2
4
6
5
15
25
35

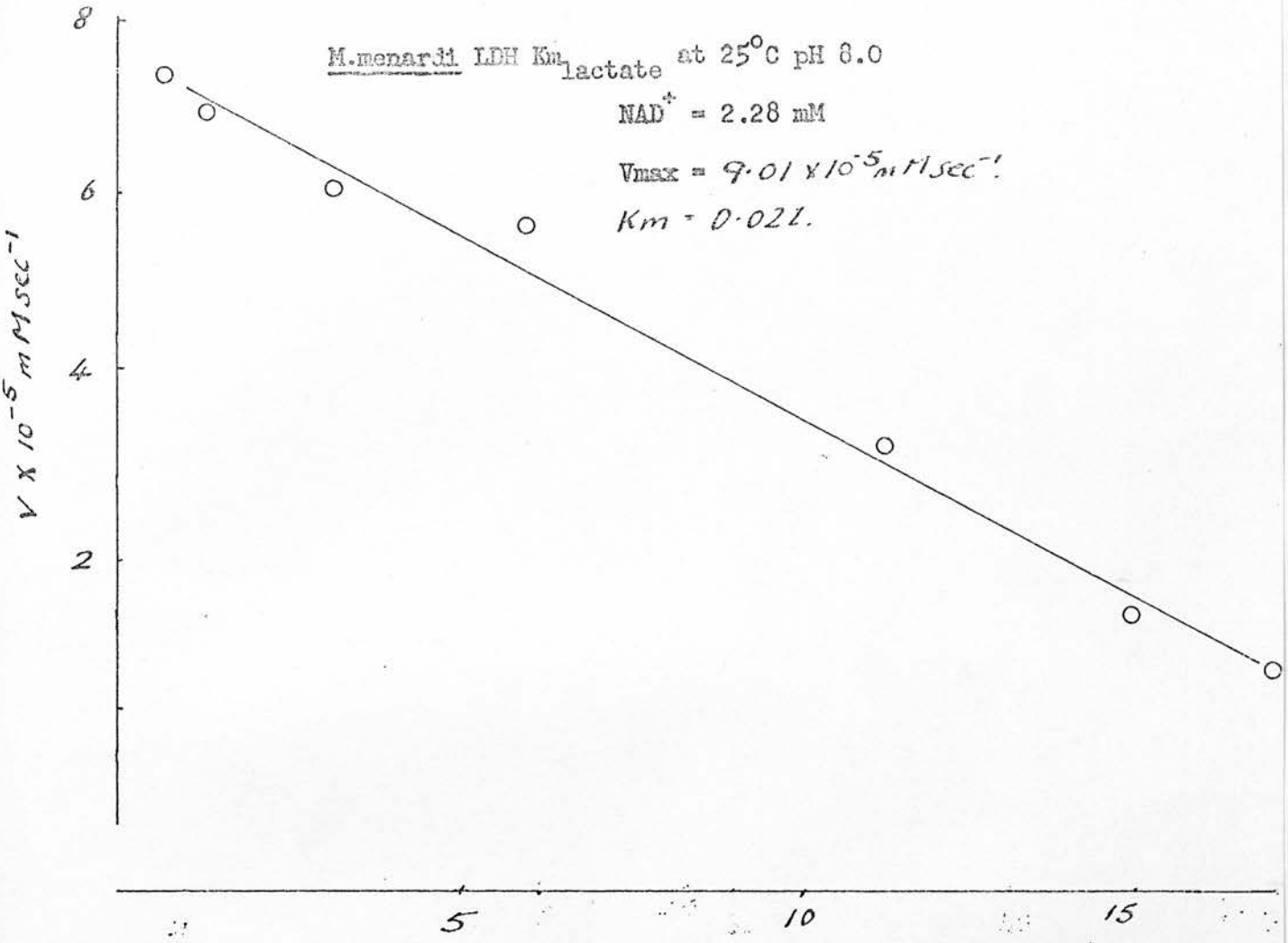

M.merianae LDH Km pyruvate at 25°C pH 8.0
NADH = 0.153 mM
Vmax = 4.86 X 10-5 mM sec-1
Km = 0.059 mM
v X 10-5 mM sec-1
v/s X 10-5 sec-1
2
4
20
35
50

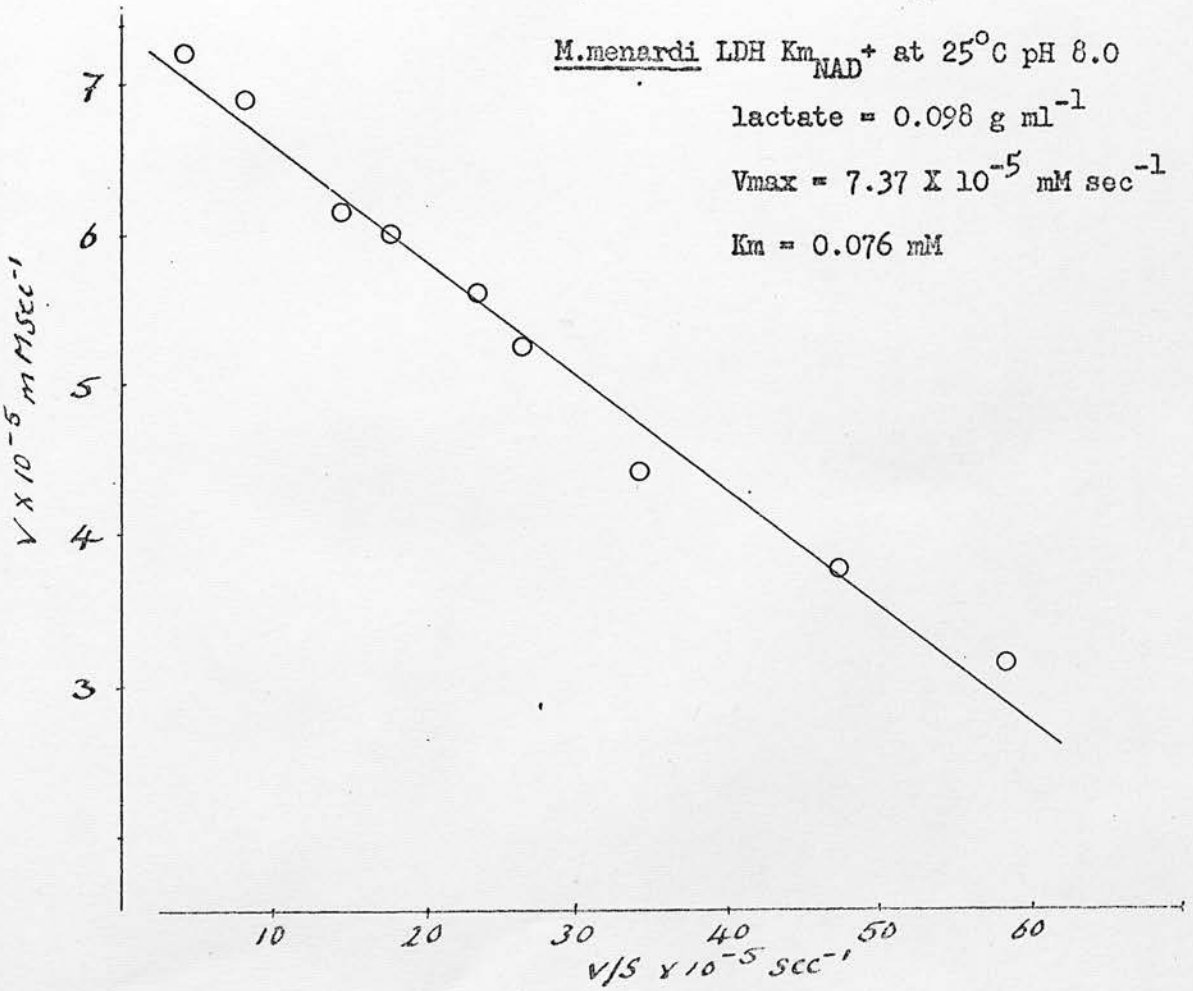

M.merianae LDH Km NAD+ at 25°C pH 8.0
lactate = 0.137 g ml-1
Vmax = 0.568 X 10-4 mM sec-1
Km = 0.115 mM
v X 10-5 mM sec-1
v/s X 10-5 sec-1


M.merianae LDH Km lactate at 25°C pH 8.0
NAD+ = 2.15 mM
Vmax = 2.53 X 10-5 mM sec-1
Km = 0.007 g ml-1
v X 10-5 mM sec-1
v/s X 10-5 sec-1


M.menardi LDH Km pyruvate at 25°C pH 8.0
NADH = 0.173 mM
Vmax = 12.36 X 10-4 mM sec-1
Km = 0.048 mM
v X 10-4 mM sec-1
12
10
8
6
4
2
4
6
8
10
12
14
v/s X 10-3 mM sec-1


M.menardi LDH Km NADH at 25°C pH 8.0
pyruvate = 0.88 mM
Vmax = 7·34 X 10-5 mM sec-1
Km = 0.018 mM
v X 10-5 mM sec-1
5
3
10
15
20
25
v/s X 10-2 mM sec-1


M.menardi LDH Km lactate at 25°C pH 8.0
NAD+ = 2.28 mM
Vmax = 9.01 X 10-5 mM sec-1
Km = 0.021.
V X 10-5 mM sec-1
8
6
4
2
5
10
15
v/s X 10-3 mM sec-1 g-1 ml.

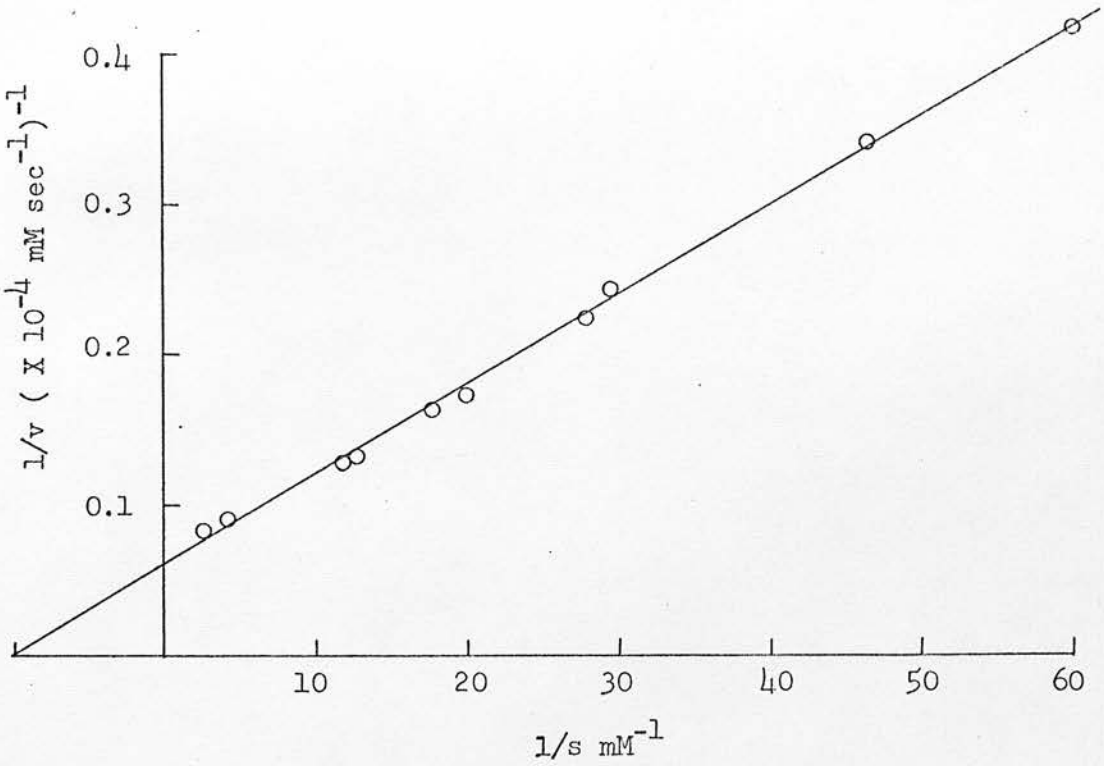

M.menardi LDH Km NAD+ at 25°C pH 8.0
lactate = 0.098 g ml-1
Vmax = 7.37 X 10-5 mM sec-1
Km = 0.076 mM
V X 10-5 mM sec-1
7
6
5
4
3
10
20
30
40
50
60
v/s X 10-5 sec-1

M.segmentata MDH $Km_{oxaloacetate}$ at 30°C, pH 7.75

NADH = 0.145 mM

Vmax = 16.13 X 10^{-4} mM sec^{-1}

Km = 0.91mM

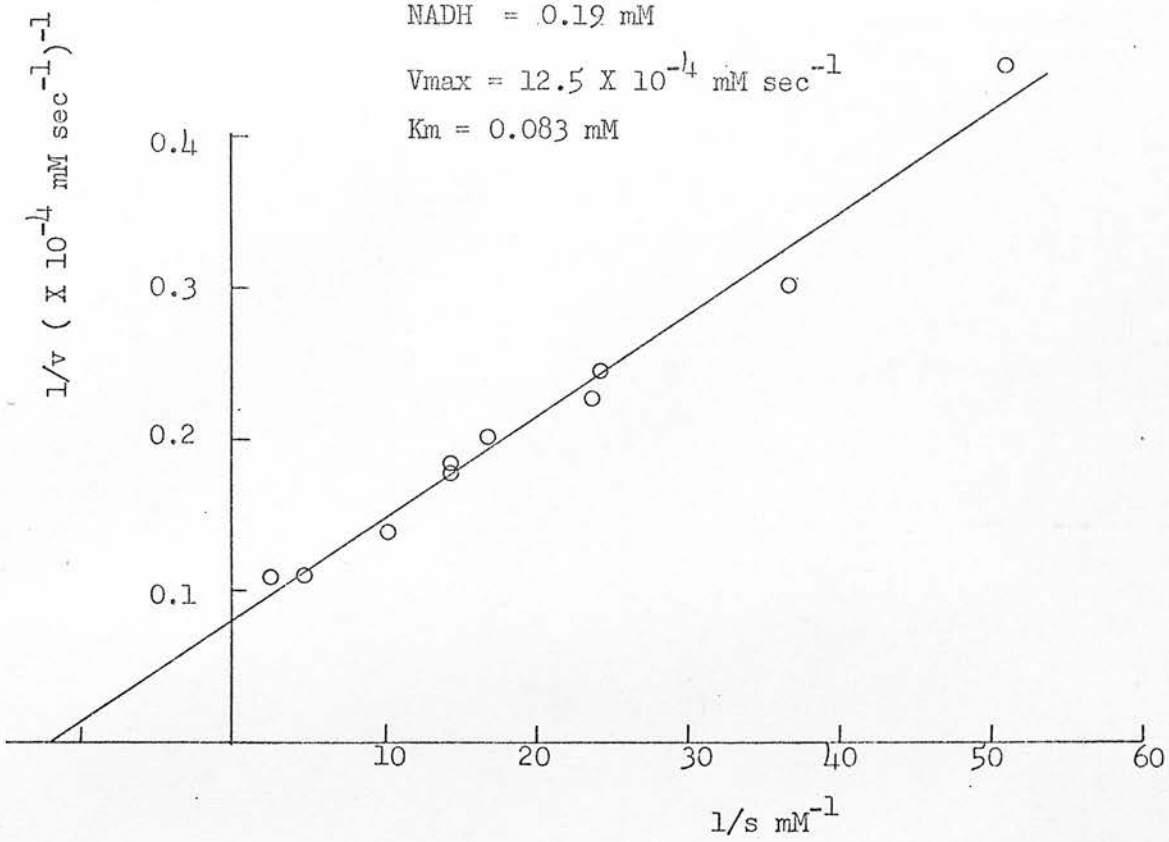


M.segmentata MDH $Km_{oxaloacetate}$ at 25°C, pH 7.75

NADH = 0.19 mM

Vmax = 12.5 X 10^{-4} mM sec^{-1}

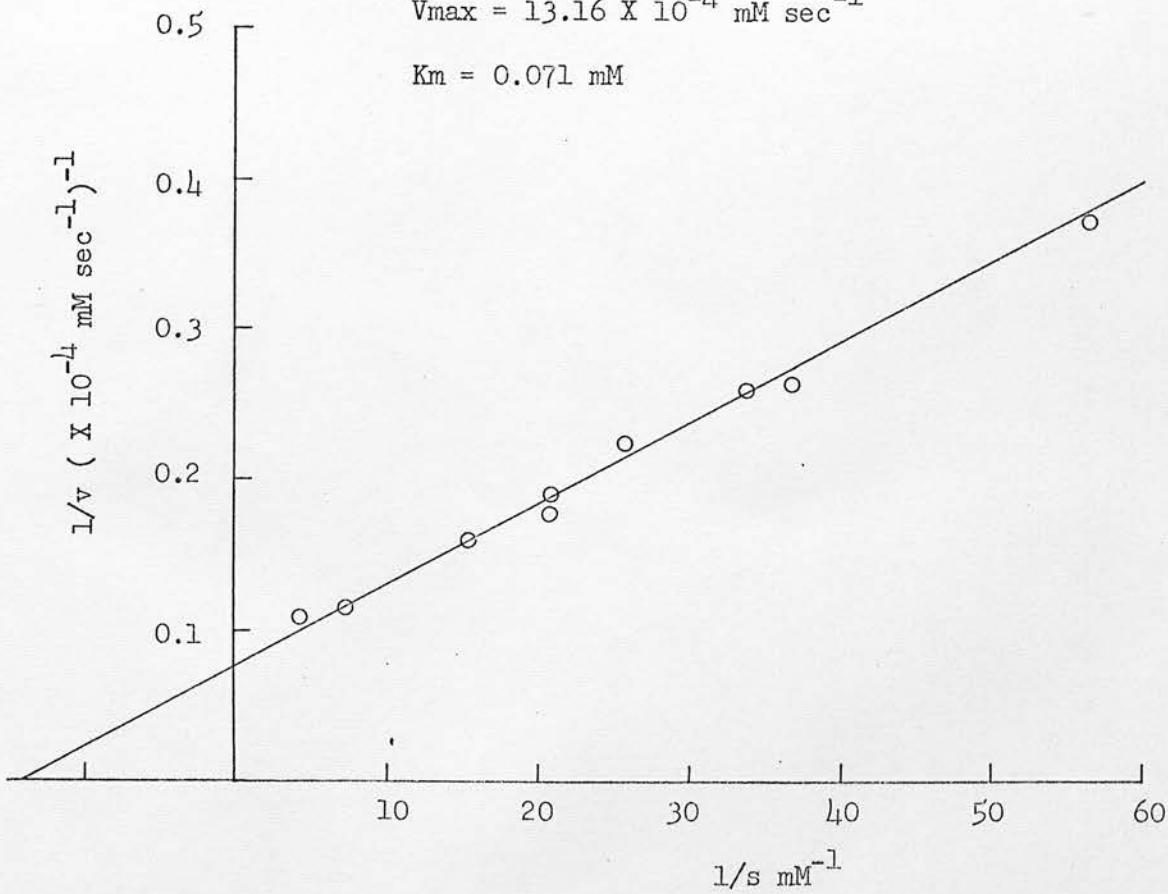
Km = 0.083 mM



(Repeat) NADH = 0.148 mM

Vmax = 13.16 X 10^{-4} mM sec^{-1}

Km = 0.071 mM

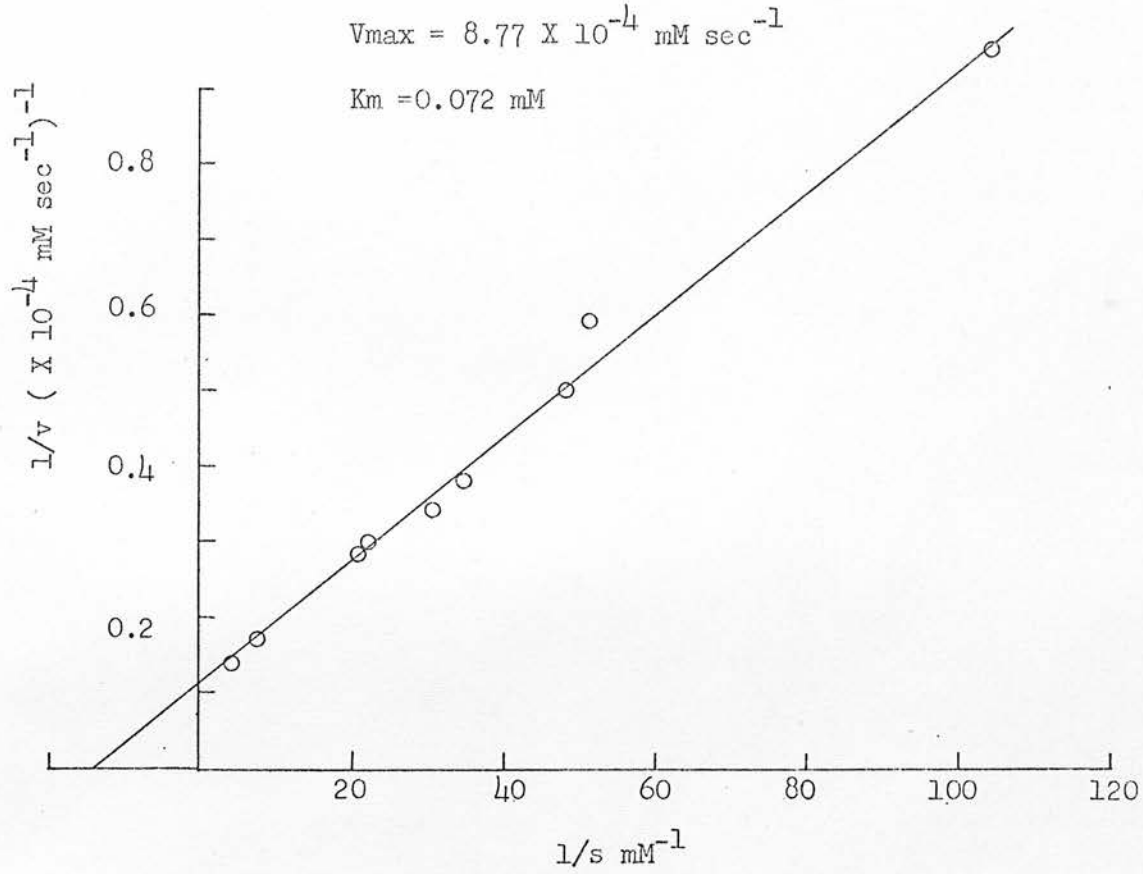


M.segmentata MDH $Km_{oxaloacetate}$ at 20°C, pH 7.75

NADH = 0.164 mM

Vmax = 8.77 X 10^{-4} mM sec^{-1}

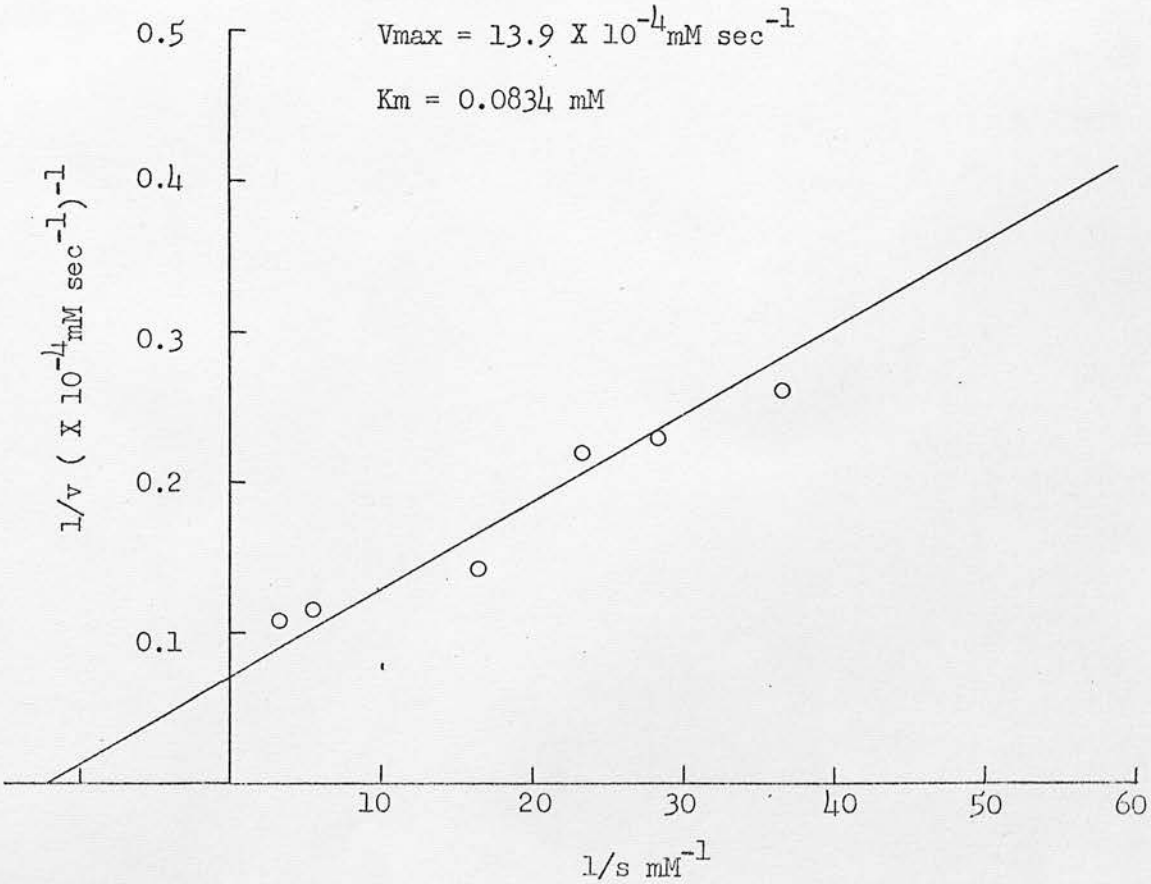
Km = 0.072 mM



(Repeat) NADH = 0.185 mM

Vmax = 13.9 X 10^{-4} mM sec^{-1}

Km = 0.0834 mM

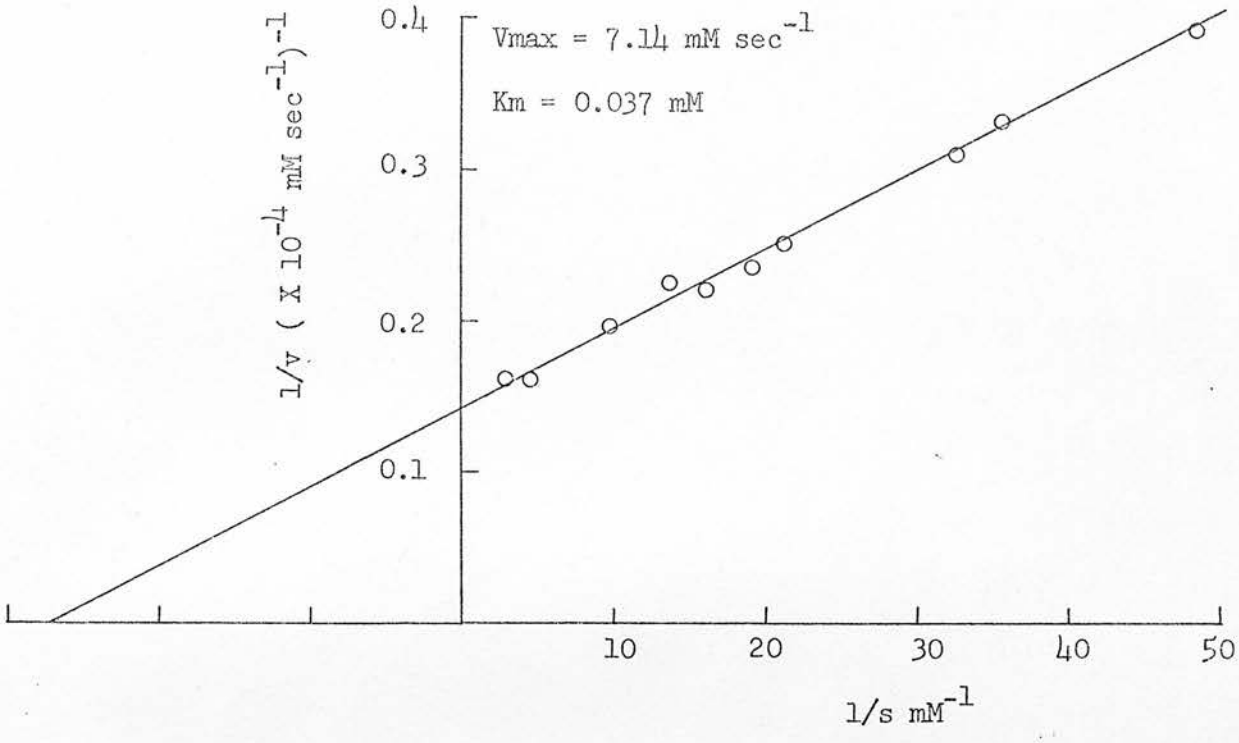


M.segmentata MDH $Km_{oxaloacetate}$ at 17.5 °C, pH 7.75

NADH = 0.138 mM

Vmax = 7.14 mM sec^{-1}

Km = 0.037 mM

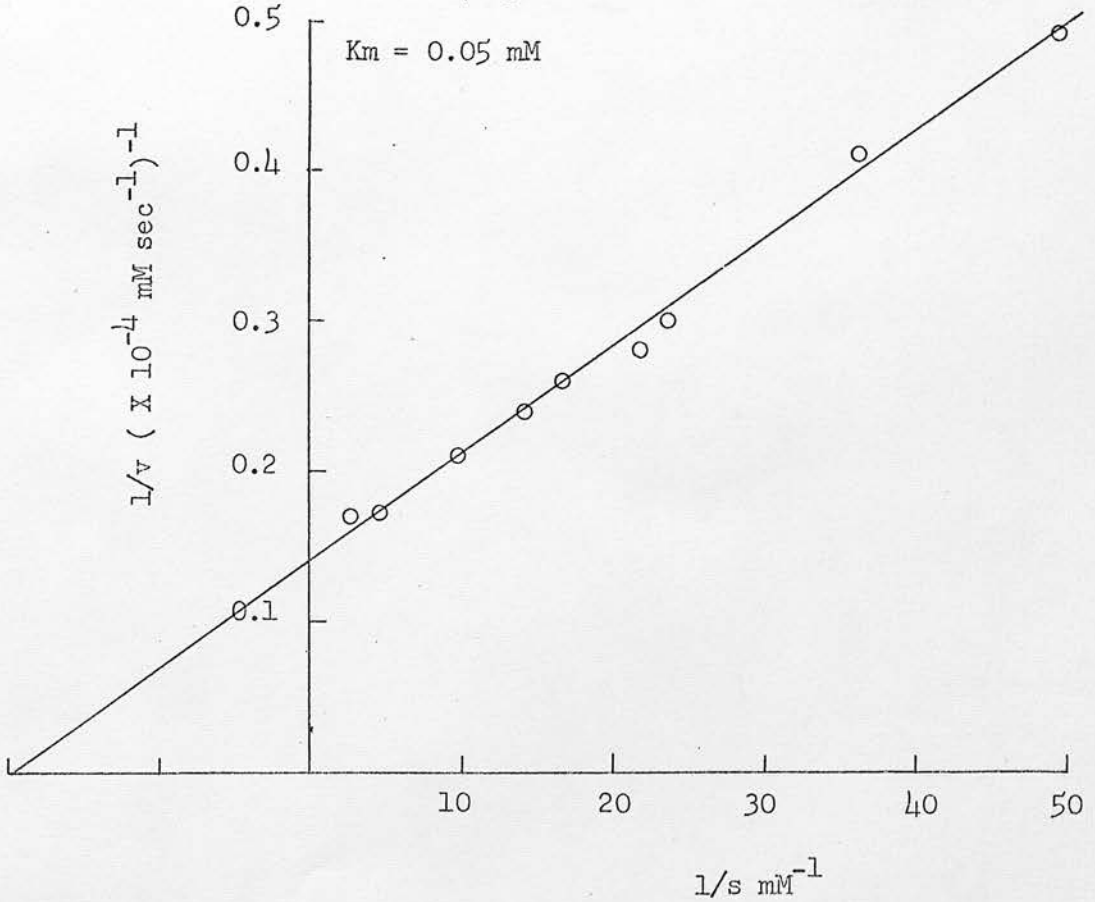


M.segmentata MDH $Km_{oxaloacetate}$ at 15°C, pH 7.75

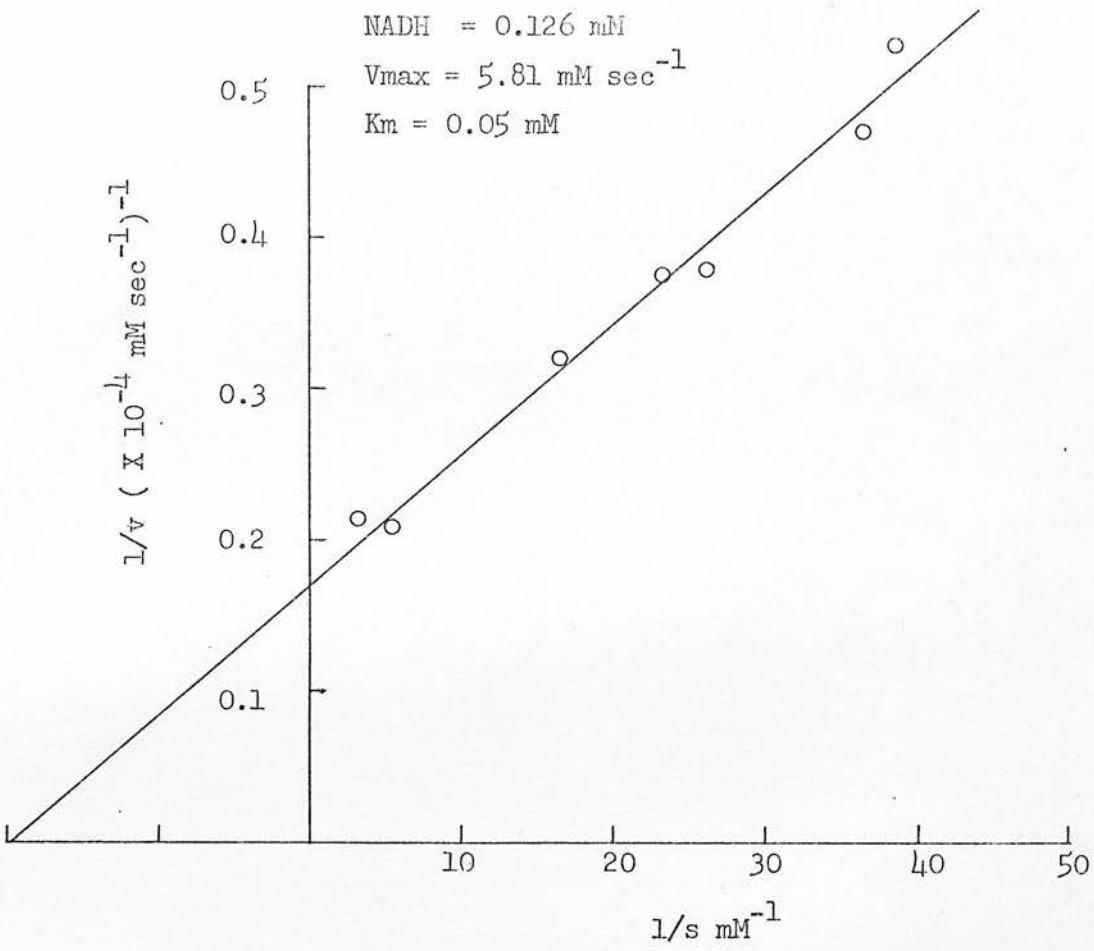
NADH = 0.121 mM

Vmax = 7.09 mM sec^{-1}

Km = 0.05 mM



M.segmentata MDH $K_{m\ oxaloacetate}$ at 12.5 °C, pH 7.75

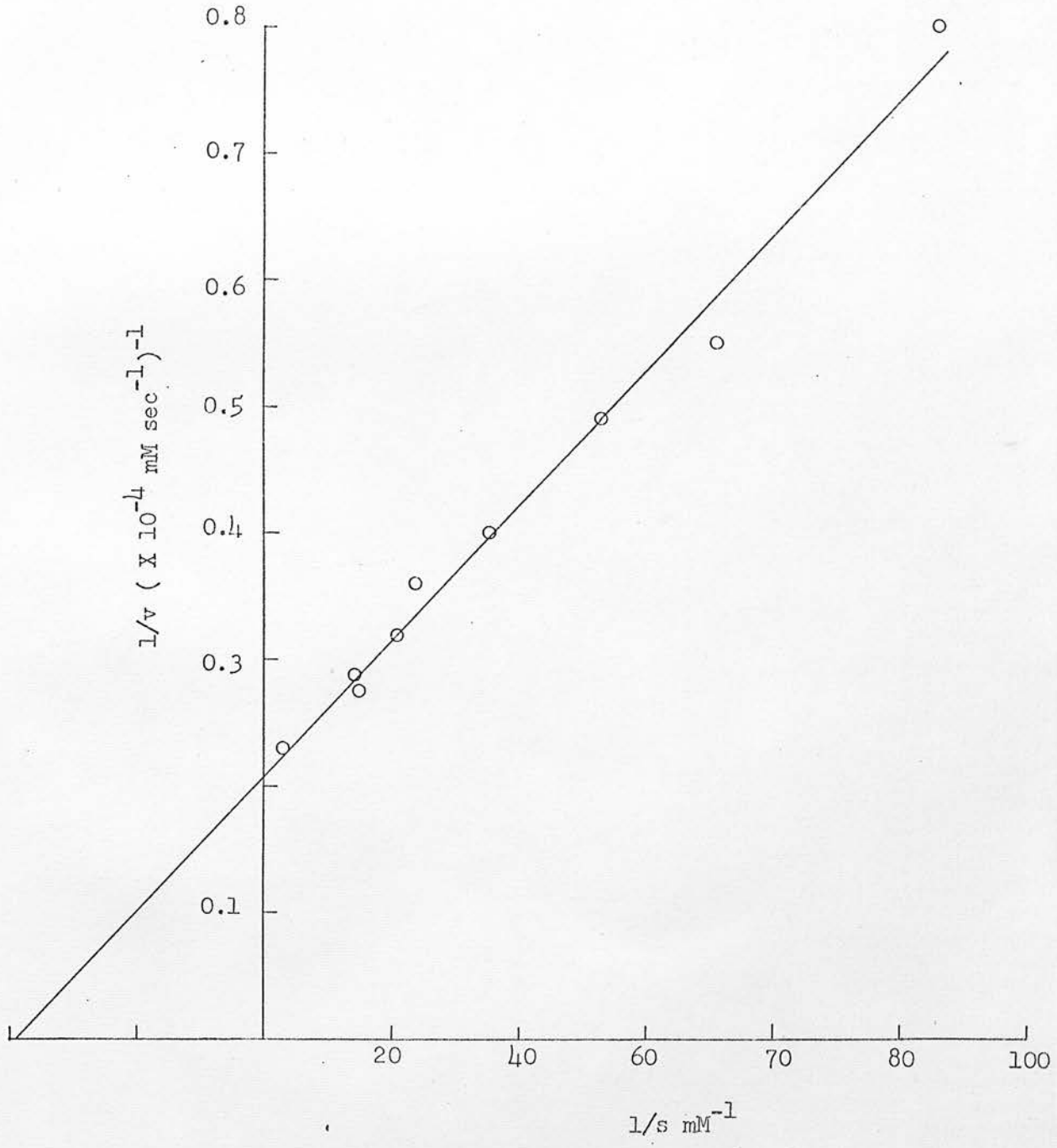


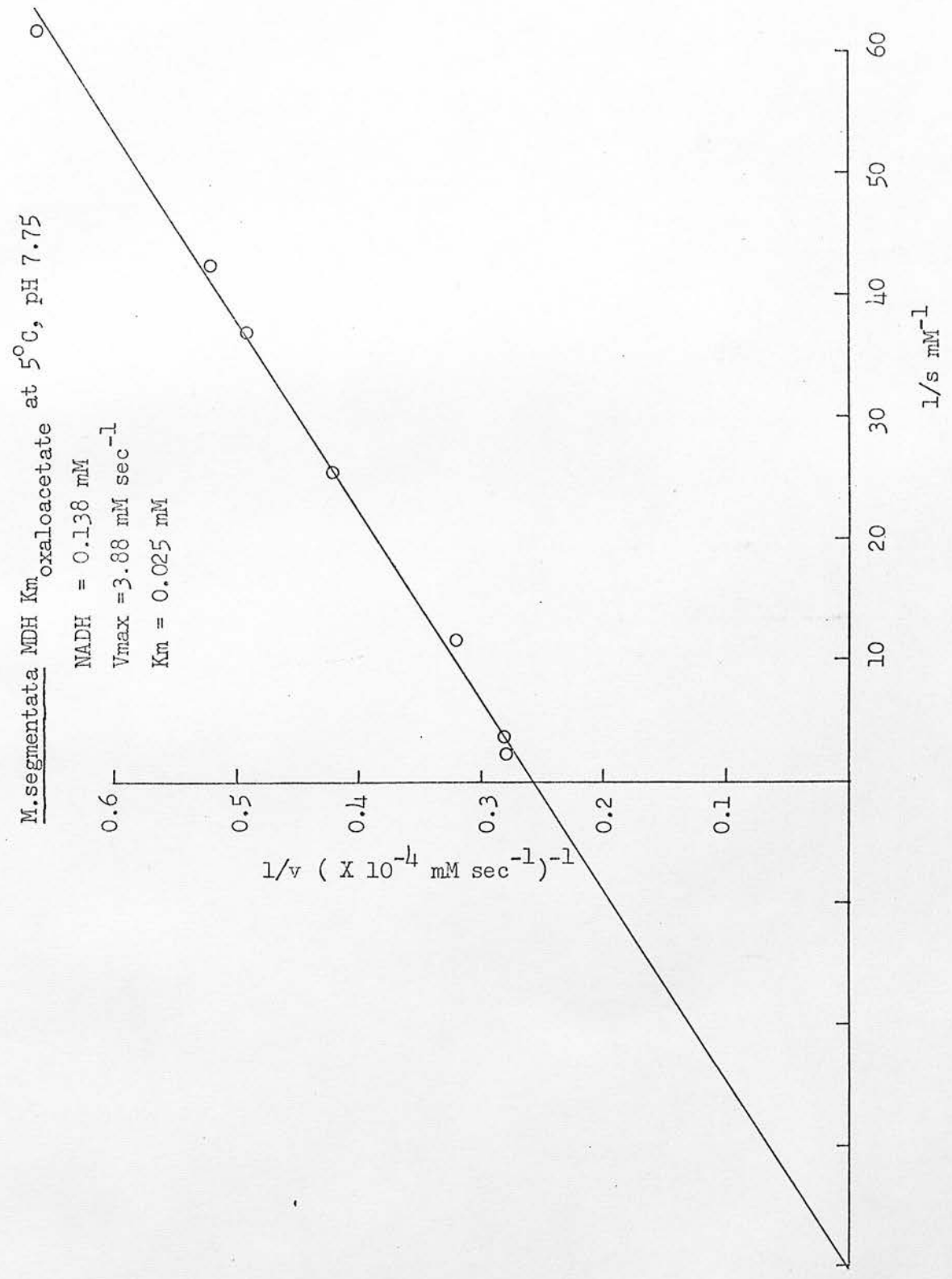
M.segmentata MDH $Km_{oxaloacetate}$ at 10 °C, pH 7.75

NADH =0.183 mM

Vmax = 4.81 mM sec^{-1}

Km = 0.051 mM




M.segmentata MDH Km oxaloacetate at 5°C, pH 7.75
NADH = 0.138 mM
Vmax = 3.88 mM sec⁻¹
Km = 0.025 mM
1/v (X 10⁻⁴ mM sec⁻¹)⁻¹
0.6
0.5
0.4
0.3
0.2
0.1
10
20
30
40
50
60
1/s mM⁻¹

Appendix 3.

Seasonal changes in size structure: M.segmentata.
Field layer sweep samples.

Collection date.	Instar mean ± S.D. cephalothorax length (mm) and % of sample in nth instar.						
	2nd	3rd	4th	5th	6th	7th	N
20 May 74	100% 0.55						2
20 May 75	100% 0.6						3
11 June 74	94% 0.61± 0.05	6% 0.78± 0.03					32
6 June 76	100% 0.63						5
Pooled mean%	94.8%	5.2					37
30 June 74	40% 0.67± 0.07	45% 0.85± 0.04	15% 1.1± 0.09				27
25 June 75	46% 0.63± 0.03	54% 0.78± 0.06					12
4 July 76	25% 0.57± 0.05	57% 0.82± 0.14		17% 1.27 (2)			12
Pooled mean%	37.9%	49.9%	7.9%	4%			51
21 July 74		45% 0.89± 0.08	45% 1.16± 0.05	10% 1.36± 0.07			17
10 Aug 74	7% 0.53 (2)	15% 0.92± 0.12	35.8% 1.19± 0.05	15% 1.47± 0.03	27.2% 1.68± 0.06		34
6 Aug 75		9% 0.89 (1)	9% 1.16 (1)	25% 1.3± 0.02	57% 1.62± 0.15		11
3 Aug 76		13% 0.81 (1)		25% 1.33 (2)	62% 1.69±		8
Pooled mean%	4%	13%	25%	17.7%	38.6%		53

M. segmentata continued

	2nd	3rd	4th	5th	6th	7th	♂ - ♀	N
5 Sept 74	8.5% 0.54 (1)		8.5% 1.22 (1)	16.5% 1.41 (2)	16.5% 1.78 (2)	50% 2.24± 0.28	0 - 6	12
28 Sept 74	25% 0.58 (1)					75% 2.15± 0.16	1 - 3	4
26 Sept 75				17% 1.36 (1)		83% 2.11± 0.26	2 -3	6
Pooled mean%	10%			10%		80%		10
22 Oct 74	9% 0.64 (1)					91% 2.13± 0.26	2 - 8	11
3 Nov 75						100% 1.84± 0.24	1 - 3	4
Pooled mean%	6%					94%		15
19 Nov 74						100% 2.24± 0.35	4 -11	15

Seasonal changes in size structure: M.mengei.

Field layer sweep samples.

Collection date.	Instar mean ± S.D. cephalothorax length (mm) and % of sample in the nth instar.							
	2nd	3rd	4th	5th	6th	7th	♂ - ♀	N
3 Mar 76		37.5% 0.66± 0.05	49% 0.84± 0.05	9.5% 1.09± 0.08	4% 1.46 (1)			27
7 Apr 74	9% 0.58± 0.05	25.2% 0.74± 0.04	23.8% 0.94± 0.05	6.5% 1.36± 0.2	1.3% 1.76± (1)	34.2% 2.1± 0.12	10-16	76
10 Apr 75		60% 0.7± 0.04	25% 0.97± 0.05			15% 2.27± 0.16	1-2	20
Pooled mean%	7.1%	32.4%	24.1%	5.1%	1%	30.2%		96
28 Apr 74	18.3% 0.6± 0.06	20.8% 0.74± 0.04	24% 0.94± 0.08	3% 1.16 (1)		34% 2.02± 0.21	12-14	80
29 Apr 75		48.4% 0.73± 0.05	32.9% 0.95± 0.04	11.9% 1.27± 0.07		8.7% 2.12± 0.24	0 - 2	23
25 Apr 76		40.5% 0.7± 0.04	32.4% 0.9± 0.05	11.7% 1.28± 0.1	5.4% 1.67± 0.13	10% 2.01± 0.55	4 - 2	60
Pooled mean%	9%	32%	28.4%	6%	2%	31.6%		163
19 May 74	6.5% 0.61± 0.06		65% 0.96± 0.14	11.5% 1.22 0.04		17% 2.03± 0.25	3 - 5	47
20 May 75		3% 0.65 (1)	60% 0.88± 0.05			37% 2.51± 0.66	5 - 2	19
Pooled mean%	4.6%	2%	63.6%	8.2%		22.8%		66
11 June 74			39% 0.94± 0.11	32% 1.25± 0.11	5.7% 1.46 (1)	23.5% 1.92± 0.15	2 - 2	17
6 June 76		3.6% 0.75 (2)	33.5% 0.94± 0.06	52.4% 1.14± 0.09	3.6% 1.42 (2)	7% 2.08± 0.13	2 - 2	56
Pooled mean%		3%	34.8%	47.7%	4%	10.8%		73

M. mengei continued

	2nd	3rd	4th	5th	6th	7th	♂ - ♀	N
30 June 74			10.7% 0.89± 0.08	65.3% 1.26± 0.09	13.4% 1.77± 0.14	10.6% 1.92 (2)	0 - 2	19
25 June 75			40% 0.91± 0.08	60% 1.23± 0.08				15
4 July 76		2.5% 0.78 (1)	34.3% 1.22± 0.08	22% 1.47± 0.05	38.7% 1.7± 0.08	2.5% (1)	0 - 1	41
Pooled mean%		1%	29.5%	40.6%	24.6%	4%		75
21 July 74	50% 0.55± 0.03			19% 1.31± 0.09	28% 1.71± 0.05	3% 1.76 (1)	0 - 1	36
10 Aug 74	55.3% 0.54± 0.03	8.2% 0.75± 0.04			35.6% 1.68± 0.18	0.9% 2.32 (1)	0 - 1	107
3 Aug 76	36% 0.55± 0.05	29% 0.76± 0.06			35% 1.75± 0.08			75
Pooled mean%	47.4%	16.8%			35.2%	0.6%		182
5 Sept 74		38.8% 0.72± 0.05	41.3% 1.0± 0.06		8.2% 1.78 (1)	16.4% 1.84 (2)	1 - 1	12
28 Sept 74	25.2% 0.59± 0.03	30.8% 0.69± 0.03		4% 1.36 (1)	4% 1.63 (1)	36% 2.22± 0.12	3 - 3	25
26 Sept 75		45% 0.7± 0.05	26% 0.9± 0.07	7% 1.16± 0.08	8% 1.65± 0.1	13.5% 2.17± 0.14	3 - 2	37
Pooled mean%	10.2%	39.3%	15.5%	5.8%	6.4%	22.6%		62
22 Oct 74		61% 0.71± 0.07	14.5% 0.96± 0.04	7.7% 1.32± 0.04	1.7% 1.75 (1)	15% 2.2± 0.2	2 - 7	60
3 Nov 75		52% 0.72± 0.07	37% 0.91± 0.05		6% 1.31± 0.07	5% 2.08± 0.42	3 - 1	76
Pooled mean%		56%	26.6%	3.4%	4.1%	9.5%		136

M. mengei continued

	2nd	3rd	4th	5th	6th	7th	♂ - ♀	N
19 Nov 74		73.5% 0.71± 0.06		20.7% 1.32± 0.08	3% 1.86 (1)	3% 2.56 (1)	1 - 0	34

Seasonal changes in size structure: M.merianae.
Field layer sweep samples.

Collection date.	Instar mean ± S.D. cephalothorax length (mm) and % of sample in the nth instar.					
	2nd	3rd	4th	5th	6th	N
29 Apr 75	55.5%	34.8%	8.7%			23
25 Apr 76	53.6% 0.6± 0.02	40.4% 0.78± 0.06		6% 1.63 (1)		31
Pooled mean%	54.4%	38%	3.7%	3.4%		54
20 May 74	85% 0.63± 0.05	15% 0.81± 0.05				11
11 June 74	41% 0.62± 0.05	51% 0.78± 0.05	8% 1.11 (1)			12
6 June 76		60% 0.73± 0.06	40% 1.11± 0.04			16
Pooled mean%	17.6%	56.1%	21.1%			28
30 June 74	9.5% 0.64± 0.05	47.5% 0.85± 0.05	38% 1.15± 0.07	5% 1.55 (1)		19
25 June 75	39.5% 0.7± 0.04	48% 0.82± 0.03	12.5% 1.18 (1)			8
Pooled mean%	18.9%	32.7%	30.4%	4%		27
21 July 74	13% 0.54± 0.07	18% 0.79± 0.06	53% 1.07± 0.07	13% 1.49± 0.09	3% 2.32 (1)	28
10 Aug 74		10% 0.7 (1)	37% 1.03± 0.07	43% 1.42± 0.04	10% 1.67 (1)	10
6 Aug 75		22% 0.94± 0.04	38% 1.09± 0.04	29% 1.47± 0.05	11% 2.4 (1)	9
Pooled mean%		15.7%	37.5%	22.6%	10.5%	19

M. merianae continued

	2nd	3rd	4th	5th	6th	N
28 Sept 76	46% 0.61± 0.04	14% 0.78± 0.05		40% 1.41± 0.1		15
26 Sept 75	23% 0.6± 0.05	25.5% 0.81± 0.05	10% 0.97± 0.04	34.5% 1.41± 0.09	10% 1.98± 0.4	29
Pooled mean%	30.8%	21.6%	6.8%	36.4%	6.8%	44
22 Oct 74	66% 0.62± 0.05	16% 0.88± 0.09		18% 1.48± 0.34		11
3 Nov 75	14% 0.6 (1)	57% 0.8± 0.04		29% 1.4± 1.11		7
Pooled mean%	46%	32%		22%		18

Seasonal changes in size structure: M.merianae.
After-dark collections.

Collection date.	Instar mean ± S.D. cephalothorax length (mm) and % of sample in the nth instar.							
	2nd	3rd	4th	5th	6th	7th	♂ - ♀	N
3 Mar 76	27% 0.6± 0.05	23% 0.79± 0.05	49% 1.42± 0.1	17% 1.86± 0.14	5% 2.43± 0.24			36
25 Apr 76	8% 0.59 (2)	29.3% 0.76± 0.05	21% 1.41± 0.4		16.5% 1.81± 0.09	25% 3.14± 1.22	3 - 3	24
6 June 76		18.4% 0.8± 0.09	36% 1.1± 0.08	13.6% 1.16± 0.16		32% 2.83± 0.87	3 - 6	28
17 June 75		22.4% 0.84± 0.06	27.2% 1.1± 0.06	18.4% 1.68± 0.16		32% 2.9± 0.9	6 - 7	41
4 July 76		5% 0.76 (2)	22.5% 1.17± 0.11	40.5% 1.46± 0.08	27% 2.2± 0.33	5% 2.56 (2)	1 - 1	38
5 Aug 75	9% 0.54 (1)		40% 1.08± 0.08	33% 1.46± 0.07	18% (2)			11
26 Sept 75	30% 0.64± 0.02	23% 0.78± 0.02	22% 1.18± 0.13		15.3% 2.01± 0.13	10% 2.88± 0.37	1 - 2	31

Seasonal changes in size structure: M.menardi.
Field layer sweep samples.

Collection date.	Instar mean ± S.D. cephalothorax length and % of sample in nth instar.				
	2nd	3rd	4th	N	
25 Apr 76	100% 0.74			2	
20 May 74	100% 0.74± 0.04			78	
20 May 75	100% 0.79± 0.05			23	
11 June 74	86% 0.81± 0.06	14% 1.08± 0.05		48	
6 June 76	68% 0.75± 0.07	32% 0.96± 0.06		15	
Pooled mean%	82%	18%		63	
30 June 74	47% 0.82± 0.05	45% 1.05± 0.05	7% 1.27± 0.06	19	
25 June 75	50% 0.81± 0.05	50% 1.08± 0.06		10	
Pooled mean%	48%	47%	5%	29	
10 Aug 74		100% 0.95± 0.01		5	

Seasonal changes in size structure: M. menardi

After-dark collections.

Collection date	Instar mean ± S.D. cephalothorax length (mm) and number collected or observed in the nth instar							
	2nd	3rd	4th	5th	6th	7th	♂ - ♀	N
25 Apr 76						1 5.3	0 - 1	1
20 May 75						obs.	1 - 2	3
6 June 76		5 0.92 ± 0.15	1 1.31			6 5.3 ± 0.21	2 - 4	12
17 June 75		28 1.07 ± 0.05				5 5.52 ± 0.31	3 - 2	33
4 July 76			9 1.42 ±0.09	Figure 1.1 1977		Figure 1.1 1977		11
3 Aug 76					1 4.5 (j♂)			1
5 Aug 75			1 1.24		1 3.44 (j♀)			2
26 Sept 76		5 1.07 ± 0.12	1 1.15			1 5.8	0 - 1	7
14 October 74						1 j♀ obs.		1